Institute of Women's Health
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Qualitative and quantitative MR imaging in preterm infants: relation to neurodevelopmental outcome

by
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To my husband Jürg and our sons Benjamin Philip and Oliver Dominik

Abstract

Despite improved survival of extremely preterm infants within the last two decades, long term cognitive, motor and behavioural impairment remain a significant burden for preterm born children. Motor impairment includes cerebral palsy (CP) and minor motor dysfunction occurring in 5-10% and 40% of very preterm born children respectively. A meta-analysis showed that children born preterm might manifest moderate to severe deficits in academic achievement, attention problems, internalizing problems and poor executive functions. Recent neuroimaging studies have helped to understand the nature of brain injuries in preterm infants. Various brain lesions occur in preterm infants, most commonly white matter (WM) injury and accompanying neuronal/axonal deficits that involve the cerebral WM, thalamus, basal ganglia, cerebral cortex, brainstem and cerebellum. However, precise prediction of outcome with conventional MRI is to date lacking. Quantitative MR has been mainly used to show normal and abnormal brain development, however few studies have tried to correlate quantitative MR at term equivalent age with later neurodevelopmental outcome. A better understanding of the mechanisms leading to neurodevelopmental impairment in preterm born children is critical in order to develop and monitor neuroprotective strategies. The aim of this thesis was to evaluate whether quantitative MRI at term corrected age is more predictive than conventional MRI of neurodevelopmental outcome in preterm infants. 80 preterm infants born < 32 weeks were recruited from the neonatal intensive care unit. MRI data was acquired on a Siemens (Erlangen, Germany) Avanto 1.5T scanner using the Siemens CP extremity coil. T1 weighted 3D-FLASH, T2-weighted fast spin echo, T2 relaxometry and diffusion weighted images were acquired. The infants were assessed at one and two years of age with Bayley III. Correlation between clinical data, conventional and quantitative MR findings and neurodevelopmental outcome at one and two years was evaluated with the focus on how quantitative MR measures might provide more information on outcome compared to conventional MRI.

WM and GM T2 values showed significant differences between preterm subgroups and between control and preterm infants with regional variation in preterm infants. T2 values correlated well with cognitive, motor and language outcome at two years of age, even after multiple corrections for clinical and MR variables. At two years of age, caudate ADC correlated significantly with language and expressive language outcome, and periventricular frontal WM ADC correlated significantly with receptive language outcome. Splenium FA correlated with cognitive, language and receptive communication outcome at two years of age. Cognitive outcome correlated with the presence of IVH, cerebellar haemorrhage, HPI and cPVL. Small CC and DEHSI were

independent risk factors for expressive language and HPI, small CC and PWML were associated with motor outcome.

Acknowledgements

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Glossary

AWM	Anterior white matter
BG	Basal ganglia
BW	Birth weight
CC	Corpus callosum
CGA	Corrected gestational age
CI	Confidence interval
CLD	Chronic lung disease
CP	Cerebral palsy
cPVL	Cystic periventricular leukomalacia
CRP	C reactive protein
CSO	Centrum semiovale
CST	Corticospinal tract
CWM	Central white matter
DCD	Developmental coordination disorder
DEHSI	Diffuse excessive high signal intensity
DTI	Diffusion tensor imaging
DWI	Diffusion weighted imaging
DWM	Deep white matter
ECS	Extracerebral space
ELBW	Extremely low birth weight
EPI	Echo planar imaging
EV	Eigenvalue
FA	Fractional anisotropy
FU	Follow up
GA	Gestational age
GM	Grey matter
GMFCS	Gross motor function Classification System
GMH	Germinal matrix haemorrhage
HFOV	High frequency ocillatory ventilation
HPI	Haemorrhagic parenchymal infarction
IHF	Interhemispheric fissure
IQ	Intelligence quotient
IVH	Intraventricular haemorrhage
M-CHAT	Modified Checklist for Autism in Toddlers
MD	Mean diffusivity
MRI	Magnetic resonance imaging
NEC	Necrotising enterocolitis
OL	Oligodendrocyte
OR	Optic radiaton
OR	Odd ratio
PDA	Patent ductus arteriosus
PLIC	Posterior limb of the internal capsule
PvWM	Periventricular white matter
PWM	Posterior white matter
PWML	Punctuate white matter lesions
RA	Relative anisotropy
RD	Radial diffusivity
ROI	Region of interest
ROP	Retinopathy of prematurity
SD	Standard deviation

SE	Spin echo
TE	Echo time
TR	Repetition time
VBM	Voxel based morphometry
VLBW	Very low birth weight
WM	White matter

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List of publications, oral presentations and posters

MRI T2 is an objective quantitative measure of cerebral white matter signal abnormality in preterm infants at term equivalent age. Hagmann CF, De Vita E, Bainbridge A, Gunny R, Kapetanakis A, Chong WK, Cady EB, Gadian DG, Robertson NJ
Radiology, 2009. 252(1): p. 209-17

Oral presentations

Preterm MRI at term: predictors of language and neurodevelopmental outcome at 2 years. CF Hagmann, R Gunny, A Bainbridge, E deVita, EB Cady, A Huertas-Ceballos, L Collier, S Johnson, N Marlow, NJ Robertson
Annual Meeting Pediatric Academic Societies' 2011, April 30-May 3, 2007, Denver, USA

Posterior white matter T2 is associated with motor and cognitive outcome at corrected age of one year. CF Hagmann, R Gunny, A Bainbridge, EB Cady, E De Vita, L Collier, A Huertas-Ceballos, NJ Robertson
Annual meeting of Pediatric Academy Society in Vancouver, 1-4 May 2010

Topographical variability of white matter abnormalities using T2 relaxometry and ADC in preterm infants at term equivalent age. Hagmann CF, E De Vita, A Bainbridge, A Kapetanakis, E Cady, S Thayyil, R Gunny, DG Gadian, NJ Robertson
48th Annual Meeting of ESPR 2007 in Prag

Quantification of diffuse white matter abnormalities using T2 relaxometry in preterm infants at term equivalent age. Hagmann CF, E De Vita, A Bainbridge, A Kapetanakis, E Cady, R Lombard, K Chong, R Gunny, DG Gadian, NJ Robertson.
ISMRM-ESMRMB, Berlin, Germany, 19-25 May 2007

Posters

White matter microstructure is associated with neurodevelopmental outcome in preterm infants at 2 years of age. CF Hagmann, R Gunny, A Bainbridge, E deVita, EB Cady, A Huertas-Ceballos, L Collier , S Johnson, N Marlow, NJ Robertson
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Quantification of diffuse white matter abnormalities using T2 relaxometry in preterm infants at term equivalent age. Hagmann CF, E De Vita, A Bainbridge, A Kapetanakis, E Cady, R Lombard, K Chong, R Gunny, DG Gadian, NJ Robertson
Annual Meeting Pediatric Academic Societies' 2007, May 3-8, 2007, Toronto, Canada

Impaired neurodevelopmental outcome associated with increased white matter Chol/Cr in preterm infants. Price D, kendall GS, Bainbridge A, Johnson S, Hagmann C, Golay X, Cady EB, Robertson NJ
Annual Meeting Pediatric Academic Societies' 2007, May 3-8, 2007, Toronto, Canada

Quantitative relationships between brain-water T2 and ADC at term in infants born prematurely. E. De Vita, C. F. Hagmann, A. Bainbridge, B. A. Kapetanakis, R. Lombard, N. J. Robertson, and E. B. Cady
ISMRM-ESMRMB, Berlin, Germany, 19-25 May 2007

T2 relaxometry in preterm infants. Hagmann CF, E De Vita, A Bainbridge, A Kapetanakis, E Cady, R Lombard, K Chong, R Gunny, DG Gadian, NJ Robertson
Prize for best poster at the Annual Academic Day of the IoWH, UCL 2006

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Chapter One

Introduction

Preterm birth <32 weeks has an incidence of 1-2% of live births (Tucker and McGuire 2004). The survival of those extremely low birth infants has been increasing over the last decade, in particular of the extremely preterm infants below 26 weeks of gestation (Lorenz et al. 1998; Hack and Fanaroff 2000; Wilson-Costello et al. 2005; Cooke 2006; Platt et al. 2007). However, despite the advances in perinatal care and thus better survival rates, many children born preterm will go on to develop major or minor motor and/or cognitive impairment. The most common major motor deficit is spastic cerebral palsy occurring in 5-10% of infants born < 32 weeks gestation. Other major disabilities include neurosensory impairments. But the majority of deficits are moderate or minor and consist of motor dysfunctions (Schmidhauser et al. 2006) and cognitive impairment (Msall et al. 1991; Saigal et al. 1991; Botting et al. 1998; Hack and Fanaroff 1999; Stewart et al. 1999; Wolke and Meyer 1999; Taylor et al. 2000; Vohr et al. 2000; Isaacs et al. 2001; Bhutta et al. 2002; Anderson and Doyle 2003; Ment et al. 2003). Periventricular white matter injury (PWMI) is the major form of brain injury. PWMI includes cystic WM injury, periventricular leukomalacia (PVL), and the diffuse WM injury. Whereas PVL was a common finding a decade ago, it accounts now for less than 5% of cases. Diffuse non-cystic WM injury is emerging to be the predominant lesion in preterm infants at term equivalent age (Counsell et al. 2003; Inder et al. 2003; Miller et al. 2003; Hamrick et al. 2004). Conventional MRI has helped to understand the nature of brain injuries in preterm infants. However, few MR studies at term equivalent age have tried to correlate MR findings with later neurodevelopmental outcome (De Vries et al. 1999; Mirmiran et al. 2004; Miller et al. 2005; Woodward et al. 2006). A recent meta-analysis tried to define the confidence in predicting neurodevelopmental outcome by MRI in preterm infants (Nongena et al.). They concluded, that moderate to severe white matter abnormalities predicted abnormal neuromotor development with a pooled probability of 35% (95% CI 19-55%) and cognitive impairment with a pooled probability of 52% (95% CI 36 - 67%)(Nongena et al.). This prediction however is based only on two studies with different white matter scoring system: the scoring system in the study by Woodward et al evaluates the extent and nature of white matter signal abnormality, the loss in volume in the periventricular white matter, the extent of any cystic abnormalities, ventricular dilatation, and thinning of the corpus callosum (Woodward et al. 2006). Miller et al classified the severity of white matter injury based on the findings of foci of

abnormal T1-weighted hyperintensity in the absence of marked T2-weighted hypointensity in the white matter; their size and localisation are scored and then graded into three severity levels (Miller et al. 2005). There are some difficulties when conventional MR studies are pooled to evaluate their predictive value: (i) few studies are published, (ii) existing studies use different scoring systems, (iii) some have included few infants and therefore have wide confidence intervals, and (iv) follow-up in these studies was limited to two years of age. Furthermore, if mild or moderate WM abnormalities are present the prediction with conventional MR proves even more difficult than for severe WM abnormalities. The study by Woodward et al reported positive predictive values of moderate to severe white matter abnormalities for abnormal neuromotor outcome of 31% with 95% CI of 17 to 49% (Woodward et al. 2006). This makes it difficult for the clinicians to counsel parents accurately. Furthermore, these scoring system are based on visual assessment of the images and hence, depending on the expertise of the assessors the grading will change and therefore the counselling.

Many research groups have focused on the development of therapeutic neuroprotective agents to prevent preterm brain injury in order to improve neurodevelopmental outcome in these infants (Volpe et al.). For such studies, a robust biomarker is required to validate the neuroprotective interventions. The National Institute of Health issued following the definitions of a biomarker: (i) a clinical endpoint which is a characteristic or a variable that reflects how a patient feels or functions, or how long a patient survives, (ii) a surrogate endpoint which is a biomarker intended to substitute for a clinical endpoint and (iii) a biological marker (biomarker) which is a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention (NIH Definitions Workgroup 2000). The use of biomarkers is advantageous especially in interventional studies as it allows for example earlier implementation of treatment (such as a neuroprotective agent) and will help to target treatment. Furthermore, biomarkers can be used if endpoints in a clinical study are very small and statistical significance might be difficult to reach. Conventional MR imaging might provide such surrogate endpoints and potentially biomarkers. However, as discussed briefly above, in preterm infants with mild to moderate brain injury, conventional MR to date is not precise enough in prediction of outcome to serve as a biomarker. Also, the prediction with conventional MR imaging is based on visual assessment, which is subjective and subject to interobserver variability and therefore not reliable enough. Quantitative MR techniques give objective measures, which can be easily reproduced and hence

those might be more reliable for prediction of outcome. Quantitative MR techniques, which have been applied in investigations of preterm brain development and injury include MR spectroscopy, diffusion tensor imaging and T2 relaxometry. They have mainly been used to show normal or abnormal brain development in preterm infants and very few studies tried to correlate quantitative MR measures with neurodevelopmental outcome.

This thesis evaluates the use of quantitative MRI in the preterm infant brain at term. The main quantitative measures I have focused on are T2 relaxometry and apparent diffusion coefficient. T2 relaxometry uses different spin-echoT2 times to yield maps of T2 relaxation times. It provides a method for investigating diffuse and discrete abnormalities that might remain undetectable on conventional MRI.

Diffusion refers to the Brownian molecular motion of the water, and can be quantified by calculating the apparent diffusion coefficient (ADC). The measurement of water diffusion in the human brain is of great clinical interest because it provides a sensitive and early indicator of brain injury. As we move towards the possibility of neuroprotection trials in the preterm infant, robust markers of outcome will be needed that accurately predict neurodevelopmental outcome; it is possible that such quantitative MR measures will be useful in such studies.

1. Background

1.1. Preterm birth

In western countries, the proportion of preterm births among live births has increased since 1980 with the highest increase in the UK (Platt et al. 2007). The incidence of preterm birth <32 weeks is 1-2% of live births in the UK (Tucker and McGuire 2004). The survival of those extremely low birth infants has been increasing over the last decade, in particular of the extremely preterm infants below 26 weeks of gestation (Fig.1.1) (Lorenz et al. 1998; Hack and Fanaroff 2000; Wilson-Costello et al. 2005; Cooke 2006; Platt et al. 2007).

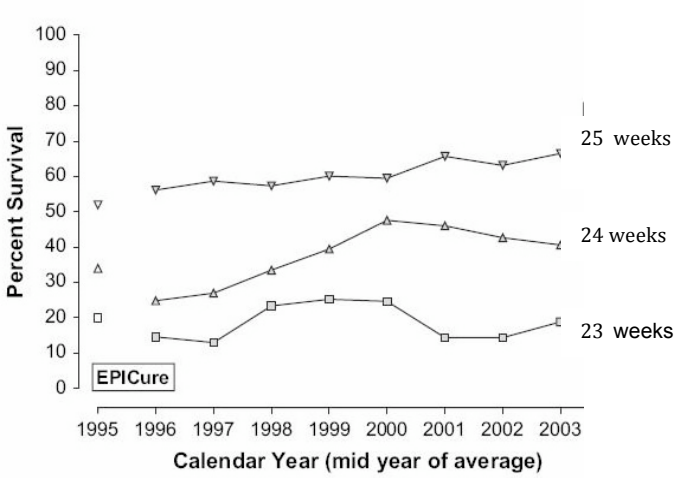


Fig.1.1. Increasing survival at borderline viability: the EPICure Study, data from March to December 1995 Costeloe et al. (2000)

A quantitative review (20 cohorts of 4116 infants of 800g or less) of mortality and morbidity in extremely preterm infants demonstrated an annual increase in survival of 2.2% for infants born <26 weeks of gestation from 1980 to 1990 and 2.1% for infants <800g from 1976 to 1990 (Lorenz et al. 1998). In a database study of 16 European centres it was shown that among infants with birth weight less than 1000g the neonatal mortality rate fell from 50% to 35%, and among those with a birth weight of 1000-1499g it fell from 20% to 5% from 1980 to 1995 (Platt et al. 2007). At UCLH there was a progressive increase in overall survival of preterm infants born <26 weeks over the last 20 years (Riley et al. 2008). The UK Epicure study reported survival to discharge at 23 weeks of 11%, at 24 weeks of 26%, and at 25 weeks of 44%; these are percentages of reported live births (Costeloe et al. 2000). A German single-centre study showed higher survival rates than the EPICure study (Kutz et al. 2009), similar to the survival rates at UCLH (Riley et al. 2008). Survival data from large population-based studies tend to show lower survival rates than from single-centre studies. However, the proportion of infants who survived without overall disability in the preterm infants born between 23 and 24 weeks was not significantly higher than in the EPICure study (Kutz et al. 2009).The NICHD network study found

that survival improved while rates of cerebral palsy remained unchanged (Tyson et al. 2008). A French observational population-based study in 1997 in nine regions of France showed that optimal care including antenatal steroids increased survival of very preterm infants but there is little evidence that this affected long-term neurodevelopmental and behavioural outcome in preterm infants born at 28 to 32 weeks and none in those born <28 weeks (Marret 2008). Hence, survival has been increased but not survival without impairment (Kutz et al. 2009; Stephens and Vohr 2009)

1.2. Preterm brain injury

1.2.1. Normal brain development

Normal brain development is divided into neuronal *proliferation, migration, organization*, and *myelination*.

The peak period for ***proliferation*** is 3-4 months of gestation with the ventricular zone (short neuronal precursor cell and radial glial cell) and subventricular zone being the sites of proliferation; proliferative units are produced by symmetrical divisions of progenitors cells and later the proliferative units enlarge by asymmetrical divisions of progenitor cells (onset of neurogenesis) before neuronal migration; hence, the general principle is the generation of neuronal units in the ventricular-subventricular zones with subsequent migration to the cortex (Rakic 1995; Volpe 2008). The radial glial cells are major neuronal progenitors for cortical neurons, astrocytes and oligodendrocytes and they guide neuronal migration (Rakic 1995; Volpe 2008).

Migration refers to the nerve cells moving from their sites of origin in the ventricular and subventricular site to the loci in the central nervous system where they reside for life. The peak period for migration is the third to fifth months of gestation. There is a radial migration (cerebral cortex-deep nuclei, projection neurons of the cortex) and a tangential migration (cerebral cortex, interneurons of the cortex). Initially, neurons migrate by translocation of the cell body; later, neurons migrate by following radial glial guides. By 20 to 24 weeks of gestation, the human cerebral cortex essentially has its full complement of neurons (Volpe 2008).

Organisation occurs in a peak time period from about the fifth month of gestation to several years after birth. The major features include i. establishment and differentiation of subplate neurons; ii. lamination; iii. synaptogenesis; iv. cell death and selective elimination of neuronal processes, and v. proliferation and differentiation of glia (Volpe 2008). The subplate is a transient developmental layer present in humans from 15 weeks to 6 months postnatally (Mrzljak et al. 1988; Kostovic and Rakic 1990). The subplate reaches its peak size and maximal

developmental impact at 24-32 weeks of gestation (Fig.1.2). The important role of the subplate neurons is to serve as sites of synaptic contact for so-called “waiting” thalamo-cortical and commissural/association cortico-cortical afferents before differentiation of the cortical plate, to serve as a functional link between these waiting afferents and their cortical targets, to provide axonal guidance into the cerebral cortex for the ascending afferents, to facilitate cerebral cortical organisation and synaptic development, and to provide pioneering axonal guidance for projections from cortex to subcortical targets (i.e. thalamus); hence, subplate neurons are central to both cortical and thalamic development (Rakic 1977; Volpe 2008) and lesions in the subplate early in the development lead to failure of these projections to reach the cortical layer (Ghosh et al. 1990). Considering that the peak of subplate formation and function correlates with the peak period of vulnerability to injury in the preterm infant and the important role of the subplate neurons in cortical development, injury to the subplate cells has been implicated in the causation of cortical deficits in preterm infants (Volpe 1996; Kostovic and Judas 2002; McQuillen and Ferriero 2004); i.e. the afferents might undergo degeneration if they did not have the subplate neurons as transient targets (Volpe 1996). Lamination and neurite outgrowth occur as neuronal migration ceases. The neurite outgrowth becomes the dominant developmental activity in the cerebral cortex: the last third trimester is a period of rapid axonal development (Kostovic and Jovanov-Milosevic 2006). The striking increase in cerebral cortical volume accompanies these developmental changes in cortical neurons. This is particularly rapid between 28 to 40 weeks of gestation (Huppi et al. 1998).

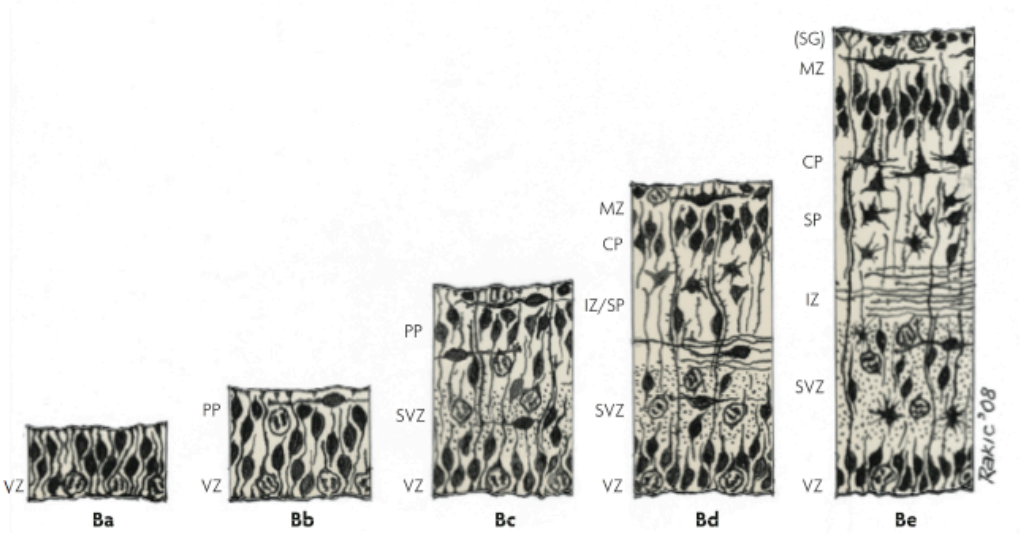


Fig.1.2. Schematic model of human neocortical development. VZ=ventricular zone; PP=preplate; SVZ=subventricular zone; IZ=intermediate zone; SP=subplate; CP= cortical plate; MZ= marginal zone; SG= subpial granular layer (Bystron et al. 2008)

Myelination is characterised by the acquisition of highly specialised myelin membrane around the axons. Myelination begins in the second trimester of pregnancy and continues into adult life. It is best considered in terms of two phases: first oligodendroglial proliferation and differentiation, and second, myelin deposition around axons (Volpe 2008). Through four basic stages of the oligodendroglial lineage the progression of the oligodendroglial lineage proceeds: oligodendroglial progenitor, pre-oligodendrocyte (O4 positive), the immature oligodendrocyte (O4 and O1 positive), and the mature myelin producing oligodendrocytes (Fig.3)(Back et al. 2007; Volpe 2008).

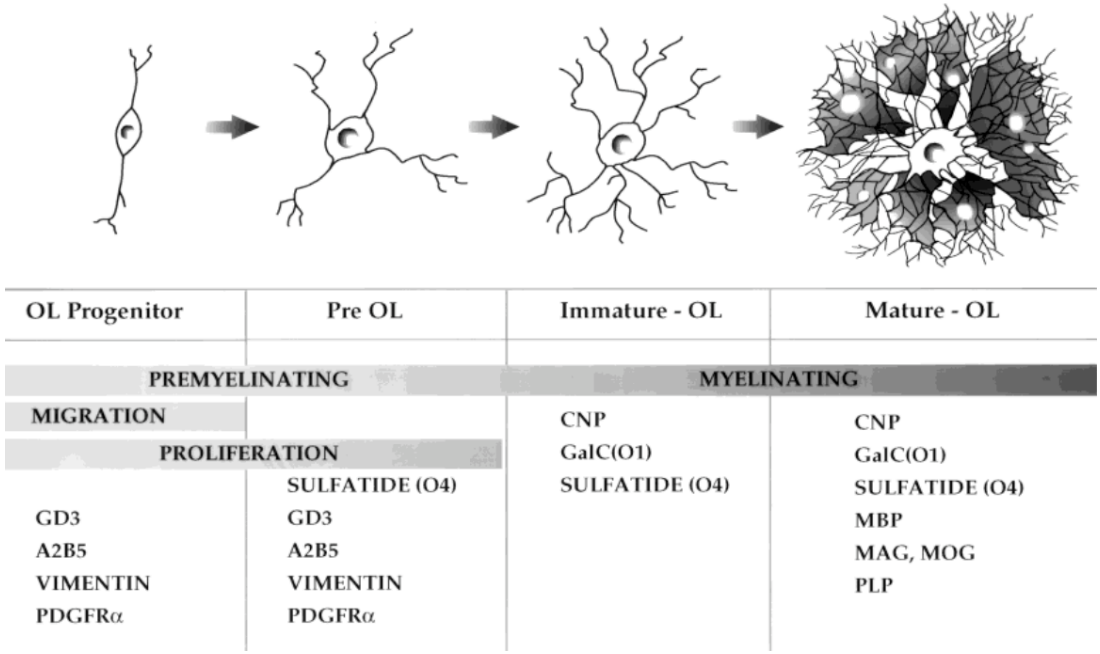


Fig.1.3. Maturation of the oligodendroglial lineage. Four principal stages of OL lineage progression are depicted together with their corresponding morphological features and capacity for myelination, migration and proliferation. Each stage is uniquely defined by a combination of marker genes or antibodies (Back et al. 2007).

Oligodendrocytes originate from the progenitors in the subventricular zone and from radial glial progenitors (Volpe 2008). The oligodendroglial progenitor is recognized by the antibodies A2B5 and NG2 (Fig.1.3 and 1.4)(Back et al. 2007). This cell is generated from midgestation to the early postnatal period. The oligodendroglial progenitor migrates into the cerebral white matter and the differentiation proceeds to the pre-oligodendrocyte, recognized by a monoclonal antibody to sulfatide (O4). The relative percentage of pre-oligodendrocytes and immature oligodendrocytes was investigated between 18 to 41 weeks: pre-oligodendrocytes were present as early as 18 weeks in the cerebral white matter and cortex and were the major oligodendrocyte

stage throughout the latter half of gestation: at 28 weeks, they comprised about 90% of the total oligodendroglial population (Back et al. 2001). Between 18 and 27 weeks, immature oligodendrocytes were a minor population, but thereafter there was an approximately threefold increase in the percentage of O1-positive cells observed at 28-41 weeks when compared with 18-27 weeks (Back et al. 2001). Hence, during 28-40 weeks of gestation, the O4-positive cells begin to differentiate to O1-positive immature oligodendrocytes, which account for about 30% of the total oligodendrocyte population during later premature period and about 50% by term. These two early differentiating forms show maturation-dependent characteristics that render them especially vulnerable to insults such as ischemia and inflammation, which leads to excitotoxicity and generation of free radicals.

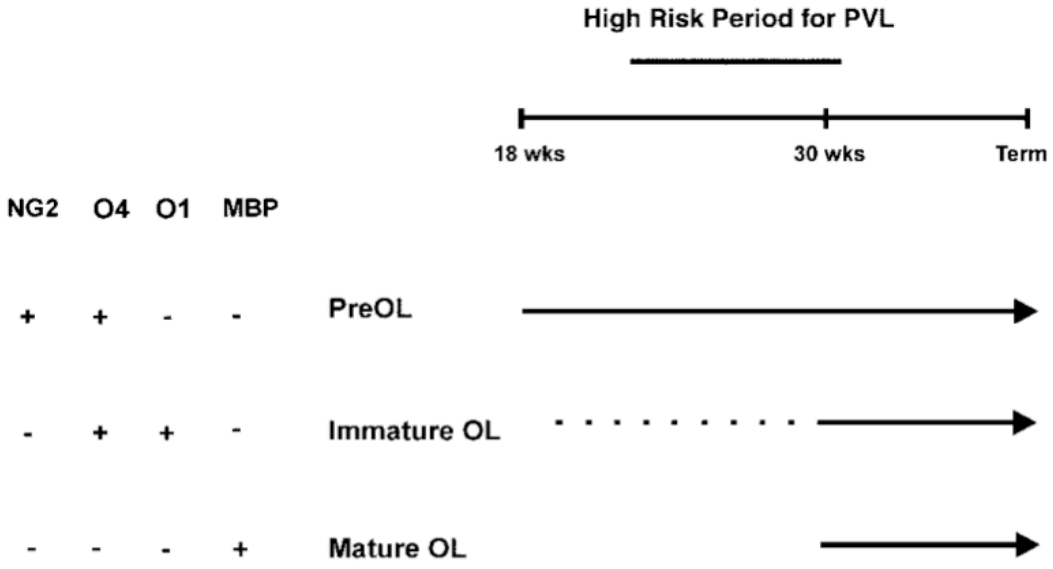


Fig.1.4. Summary diagram of oligodendrocyte (OL) lineage progression in human cerebral white matter during latter half of gestation. The high-risk period for periventricular white matter injury coincides with the developmental epoch when the white matter is mainly populated with O4+O1-pre-oligodendrocytes (Back et al. 2001)

Pre-oligodendrocytes and immature oligodendrocytes ensheath axons in preparation for full differentiation to myelin-producing oligodendrocytes (Fig.1.5).

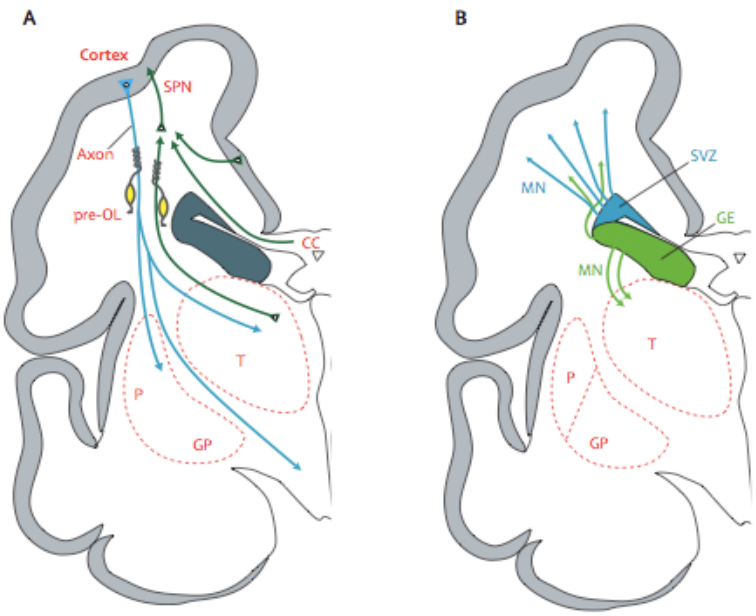


Fig.1.5. A. Axons (green) emanate from thalamus (T, projection fibres), corpus callosum (CC, commissural fibres) and cortex (association fibres). Subplate neurons (SPN) send axons to cortex. From cortex axons (blue) descend to thalamus, basal ganglia and corticospinal tracts. Pre-oligodendrocytes ensheath axons before full differentiation to mature myelin-producing oligodendrocytes. B. Proliferation and migration (MN) of interneurons from subventricular zone (SVZ) and ganglionic eminence (GE) (Volpe 2009)

Differentiation of oligodendroglia has been shown to depend closely on the presence and integrity of axons and it has also been shown that a minimal axonal diameter is important for the initiation of myelination (Van der Knaap 2005). The myelin sheath needs an intact axon as is demonstrated by Wallerian degeneration. On the other hand, the axon requires an intact myelin sheath for maintenance of normal structure and function. Axonal development is pronounced in the last trimester of gestation and in early postnatal period (Kostovic and Jovanov-Milosevic 2006).

***Progression of myelination**:* There is marked temporal diversity in topographic patterns of myelination throughout the last half of gestation and during the first two postnatal years. Hence, at any time in early development of the human brain there are multiple separate or intermixed regions of unmyelinated, myelinating and myelinated white and grey matter.

- In the *fourth* month of gestation, myelin is first seen in the anterior motor roots and soon appears in the posterior roots.
- In the *fifth* month of gestation, myelination starts in the dorsal columns of the spinal cords and the anterior and lateral spino-thalamic tracts.

- In the *sixth* month of gestation myelination progresses rapidly cephalad in the medial lemniscus and spino-thalamic tracts in the brain stem tegmentum. Myelin begins to appear in the stato-accoustic tectum, tegmentum, lateral lemniscus and in the inner, vestibulo-cerebellar part of the inferior cerebellar peduncle.
- In the *seventh* month of gestation, myelin is largely confined to structures outside the diencephalon and cerebral hemispheres. Progression is seen in the optic nerve, chiasm and tracts, inferior cerebellar peduncles, vestibulo-spinal, reticulo-spinal and tecto-spinal descending tracts to the spinal cord and posterior limb of the internal capsule.
- In the *eighth* month, myelination starts in the corpus striatum, anterior limb of internal capsule, subcortical white matter of the post-and precentral gyri, rostral part of the optic radiation as well as the cortico-spinal tract in midbrain and pons, middle cerebellar peduncles and cerebellar hemispheres.
- In the ninth month of gestation, it continues in the thalamus, putamen, central part of the coronal radiata, distal part of the optic radiation, anterior commissure, midportion of the corpus callosum and fornix.

At term, apart from some myelin in the central tracts of the corona radiata connected to the pre-and postcentral gyri, and the primary optic and acoustic radiations, the cerebral hemispheres are largely unmyelinated (Van der Knaap 2005). In 1901, Flechsig described a temporal sequence of cerebral myelination indicating that the posterior white matter, beginning around the central sulcus and in the occipital lobe, generally myelinates before the anterior white matter (Flechsig 1901). In 1967, Yarkolev and Lecours published a diagram of progression of myelination (Fig.1.6.) (Yakolev and Lecours 1967).

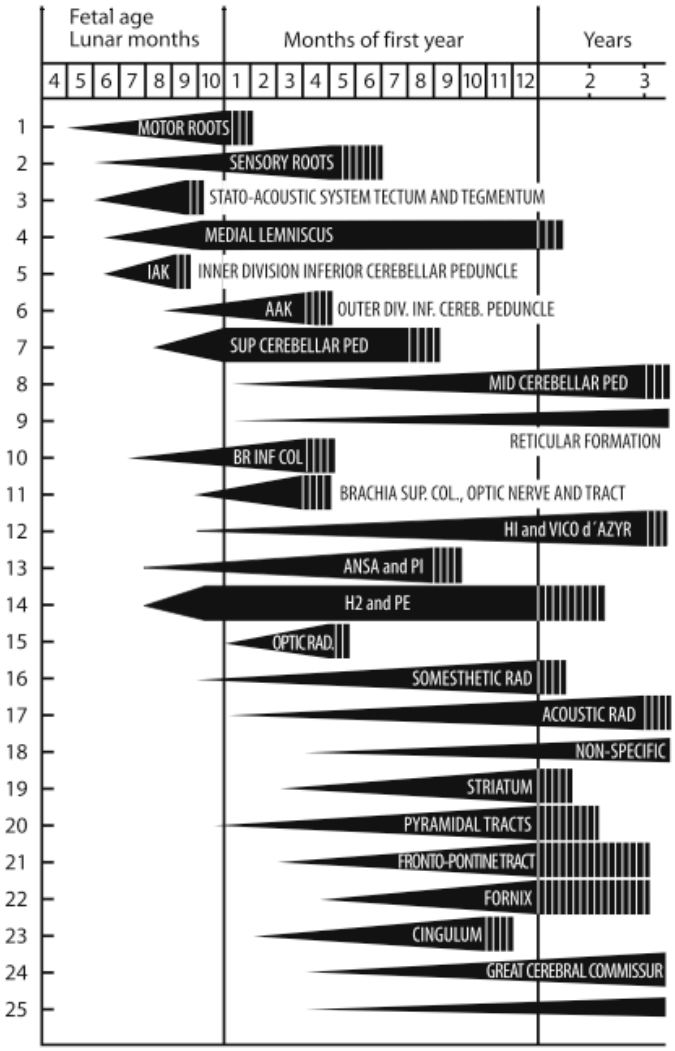


Fig.1.6. Classic diagram of progression of myelination as conceived by Yakolev and Lecours (1967). Appearance of myelination on MR images is 1 or 2 weeks behind this schedule with conventional MRI

In 1988, based on precisely defined white matter sites in 162 autopsied children, Kinney et study described that myelination in telencephalic sites progresses from the central sulcus outwards toward the poles and that the occipital pole myelinates before the frontal pole which in turn myelinates before the temporal pole (Fig.1.7) (Kinney et al. 1988). In addition, the posterior hemispheres (posterior fronto-parieto-occipital regions) have shorter myelination intervals than the anterior fronto-temporal regions; hence, the posterior limb myelinates earlier and faster than the anterior limb of the internal capsule, and the body and splenium of the corpus callosum earlier than the rostrum (Kinney et al. 1988).

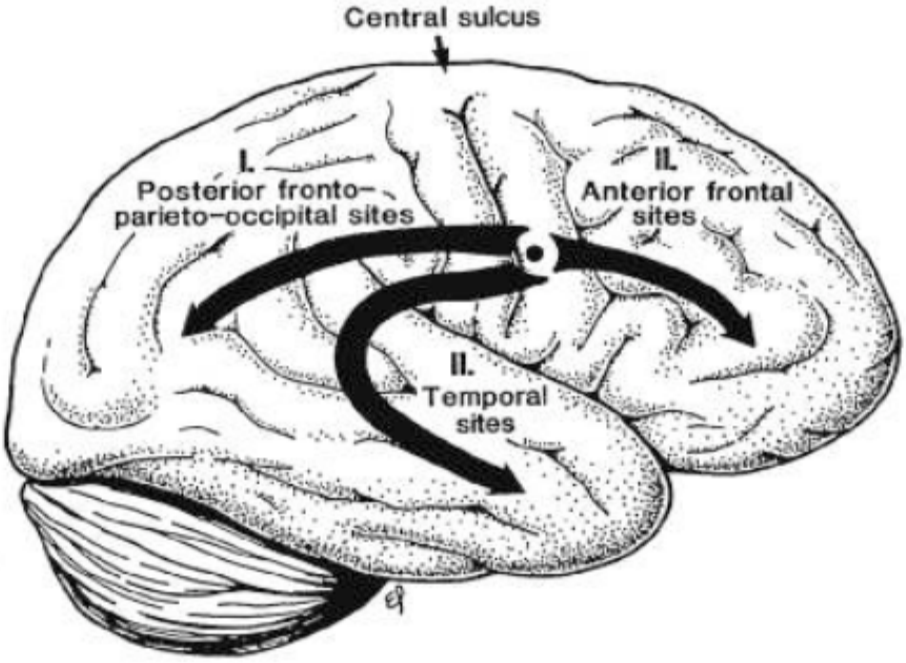


Fig.1.7. Drawing of the cerebrum depicting the progression of myelination in telencephalic sites from the central sulcus outward to the poles, with the posterior sites preceding the anterior fronto-temporal sites (Kinney et al. 1988).

Five major rules concerning cerebral myelination can be derived from this study (Kinney et al. 1988):

i. Proximal pathways myelinate before distal pathways,
ii. Sensory pathways myelinate before motor pathways
iii. Projection pathways myelinate before cerebral associative pathways
iv. Central cerebral sites myelinate before cerebral poles
v. Occipital poles myelinate before frontotemporal poles

1.2.2. Preterm brain injury

Various brain lesions occur in preterm infants, most commonly white matter injury and accompanying neuronal/axonal deficits that involve the cerebral white matter, thalamus, basal ganglia, cerebral cortex, brainstem and cerebellum. Severe germinal matrix-intraventricular haemorrhage, particularly haemorrhagic parenchymal infarctions are important lesions but occur less frequently. The emphasis of this subsection will be on diffuse white matter injury and the accompanying neuronal/axonal deficits.

1.2.2.1. White matter injury

Periventricular white matter injury (PWMI) is the major form of brain injury and the leading cause of neurodevelopmental impairment in children born preterm. PWMI includes cystic WM injury, periventricular leukomalacia (PVL), and the diffuse WM injury (Fig.1.8). Commonly, PWMI presents as symmetrical lesions localised adjacent to both lateral ventricles, with regions of particular predilection for injury being anterior or lateral to the anterior horns and lateral to the trigone and posterior horns. PVL might be accompanied by diffuse white matter injury or might occur as an isolated lesion

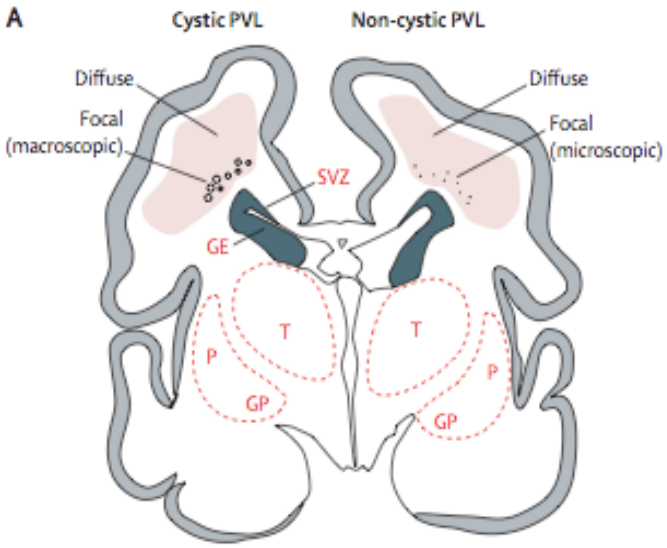


Fig.1.8. Coronal sections of a 28 weeks old preterm infant showing the focal (small circles) and diffuse (black dots) white matter injury (Volpe 2009)

Whereas PVL was a common finding a decade ago, it accounts now for less than 5% of cases. These PVL cysts are macroscopic, evolve over several weeks and can be easily seen by cranial ultrasound. Diffuse non-cystic WM injury is emerging to be the predominant lesion in preterm infants at term equivalent age.

Pre-oligodendrocytes seem to be the main cellular target in diffuse white matter injury; this has been shown in human (Haynes et al. 2003; Back et al. 2005; Robinson et al. 2006) and experimental models (Back et al. 2002; Back et al. 2005; Volpe 2008).

In the early stages of injury, diffuse PWMI is distinguished by the presence of numerous reactive microglia in the periventricular white matter and by a decrease in pre-myelinating oligodendrocytes (Kinney and Back 1998; Haynes et al. 2003; Back et al. 2005). At later stages, diffuse PWMI lesions contain numerous reactive astrocytes (diffuse gliosis) (Leviton and Gilles 1984; Haynes et al. 2003).

Pathogenesis of diffuse white matter injury

The principal initiating pathogenic factors in white matter injury appear to be cerebral ischemia and maternal/neonatal infection and fetal inflammation. These upstream mechanisms activate two critical downstream mechanisms that are excitotoxicity and free radical attack by reactive oxygen and nitrogen species that lead to death of the pre-oligodendrocytes (Fig.1.9).

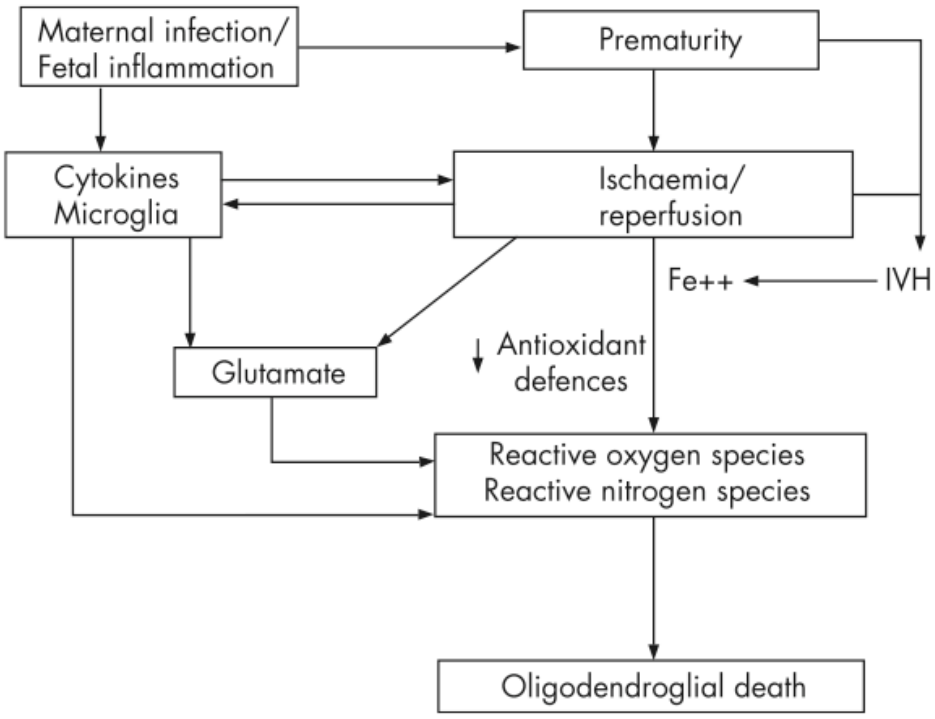


Fig.1.9. Pathogenetic mechanisms in PVL (Khwaja and Volpe 2008)

i. Vascular theory (upstream mechanism)

There is a hypothesis relating to the presence of apparent vascular end zones in the periventricular white matter that are supplied by long and short penetrator (Fig.1.10) (Volpe 2001). In the setting of a pressure passive circulation, these vascular end zones may be particular susceptible to ischemia (Volpe 2001). This hypothesis proposes that deep-seated focal cystic necrosis lesions of PVL arise from severe or persistent ischemia in vascular end zones of long penetrators (Nakamura et al. 1994). Less severe or briefer episodes of ischemia in the territory of more superficially situated short penetrators may account for the more extensive

myelination disturbances in diffuse white matter injury (Volpe 2001).

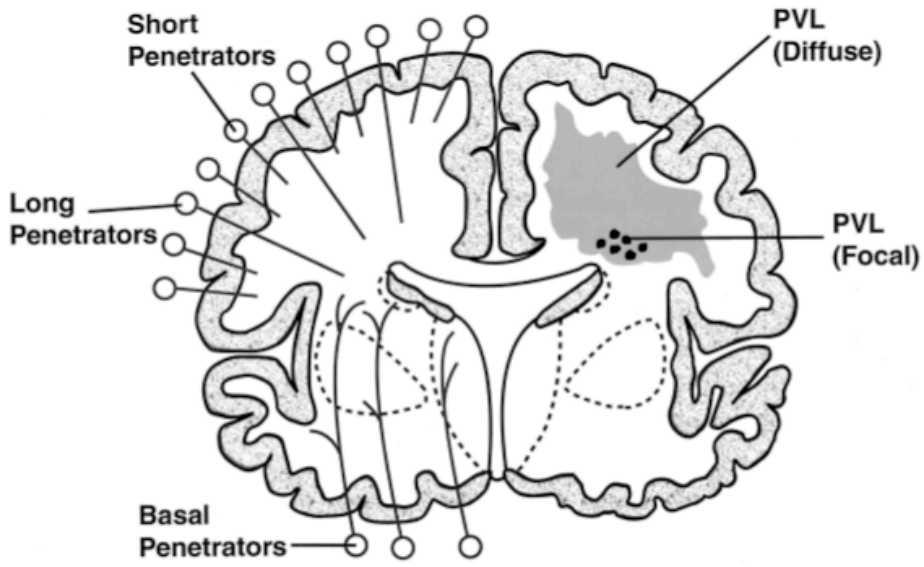


Fig.1.10. Schematic depiction of coronal section of cerebrum with focal and diffuse components of PVL in one hemisphere and the cerebral vascular supply in the other hemisphere (Volpe 2001)

However, direct experimental evidence that human periventricular white matter is selectively susceptible to ischemia is lacking. One problem is that current measures of global cerebral blood flow lack the spatial resolution to define cerebral hemodynamics in human periventricular white matter. Using near infrared spectroscopy, it was found that impaired cerebrovascular autoregulation correlated with the development of PVL and germinal-matrix haemorrhage (Tsuji et al. 2000). In recent experimental work in immature fetal sheep, the duration of cerebral ischemia was found to be a critical factor in the generation of a graded spectrum of white matter injury (Riddle et al. 2006); ischemia of 30 to 37 minutes duration led to selective graded injury to frontal and parietal periventricular white matter whereas 45 minutes of ischemia generated damage to cortical and subcortical white matter (Back et al. 2007). Interestingly, the medial and lateral periventricular white matter sustained differing degrees of acute injury even though they sustained a similar degree of low flow during prolonged severe ischemic insult. Hence, no regional differences in blood flow were found within periventricular white matter to account for the disparate rates of cellular degeneration in medial and lateral periventricular white matter (Back et al. 2007). Indeed, the predilection for periventricular white matter injury was closely related with the distribution of pre-oligodendrocytes. Hence, these findings suggest that perturbations in cerebral blood flow are necessary but not sufficient to damage periventricular white matter (Back et al. 2007).

The developmental predilection for periventricular white matter injury to occur during prematurity appears to relate to both the timing of appearance and regional distribution of susceptible pre-oligodendrocytes (Riddle et al. 2006; Back et al. 2007).

ii. Infection/Inflammation (upstream mechanism)

A number of epidemiological studies have shown an association between maternal/fetal infection and sonographically detectable PVL or cerebral palsy (Leviton et al. 1999; Leviton et al. 2005). Both outcomes are increased in the presence of infection of the decidua, placenta, amniotic fluid, fetal vasculitis, raised cytokines in the neonatal blood, and intrauterine T cell activation (Wu and Colford 2000; Duggan and Edwards 2001; Duggan et al. 2001; Kaukola et al. 2006). But chorioamnionitis is strongly associated with preterm delivery and the sequence of causation is not clear (Wu 2002). Preterm infants with neonatal sepsis have increased rates of cerebral palsy and PVL (Inder et al. 2003; Stoll et al. 2005). Kaukola et al showed a high risk for abnormal neurological outcome in histological chorioamnionitis and placental perfusion defect; they proposed that chorioamnionitis together with other insults such as placental perfusion defect or maternal systemic infection increase the risk for poor neurological outcome (Kaukola et al. 2006). Most recently, a direct relationship between recurrent infection and progressive postnatal white matter injury diagnosed by MR imaging has been shown (Glass et al. 2008). Postnatal infection could be an important factor in white matter injury and an important target for prevention.

Animal studies support the hypothesis that perinatal infection causes cerebral injury by inflammatory mechanisms, perhaps in synergy with other factors such as hypoxia-ischemia. Indeed, fetal and neonatal infection can be associated with persistent hypotension and impaired cerebrovascular autoregulation (Yanowitz et al. 2004; Yanowitz et al. 2006). Potentiation of infection/inflammation and ischemia have been shown in animal studies in which pretreatment with lipopolysaccharides (LPS) caused a subthreshold hypoxic-ischaemic insult to produce severe injury (Eklind et al. 2004). LPS activates the innate immune system through interaction with specific toll-like receptors on immune cells, including microglia, and secretion of molecules directly toxic to pre-oligodendrocytes (Lehnardt et al. 2002).

iii. Free radical attack (downstream mechanism)

Free radical attack appears to be the principal final common pathway to injury. Free radical attack seems to lead to death of pre-oligodendrocytes but not of reactive astrocytes: in a study of 17 cases, in which immunocytochemical markers for

oxidative and nitrative attack were used, abundant staining was documented in both pre-oligodendrocytes and reactive astrocytes in the diffuse lesions (Haynes et al. 2003). This is consistent with experimental data showing oxidative and nitrative cellular injury with both hypoxic-ischaemic and inflammatory insults to brain and a selective vulnerability of pre-oligodendrocytes to ROS/RNS (Volpe 2001). In recent studies, marked microgliosis has been seen in diffuse white matter injury (Haynes et al. 2003): hypoxia-ischemia and infection both can activate microglia leading to their activation and secretion of toxic products, especially ROS/RNS, and death of pre-oligodendrocytes. Human and experimental studies indicate a maturation-dependent window of vulnerability to oxidative attack during oligodendroglial development related principally to delayed development of antioxidant enzymes and acquisition of iron for differentiation (Khwaja and Volpe 2008).

iv. Excitotoxicity (downstream mechanism)

Glutamate is capable of inducing maturation-dependent death of pre-oligodendrocytes by non-receptor and receptor-mediated mechanisms. Receptor-mediated glutamate toxicity is the principal mode of excitoxicity and pre-oligodendrocytes contain glutamate receptors (AMPA/kainate and NMDA) which, when excessively activated, lead to cell injury (Itoh et al. 2002; Rosenberg et al. 2003). This toxicity is maturation-dependent and both functional activity and subunit expression of AMPA/KA receptors are up-regulated in pre-oligodendrocytes rather than in mature oligodendrocytes (Rosenberg et al. 2003). The pre-OL are particularly susceptible to glutamate-mediated receptor toxicity and the timing of glutamate receptor expression coincides with the window of diffuse white matter injury vulnerability (Follett et al. 2004). The mechanism for the receptor-mediated toxicity appears to be Ca^{2+} influx (Rosenberg et al. 2003). Knowledge of the mechanism leading to cell death gives the opportunity to develop preventive interventions such as block of AMPA receptors (topiramate), and block of NMDA receptors (memantine).

1.2.2.2. Neuronal/axonal disease (Fig.1.11) (Volpe 2009)

Pre-oligodendrocyte injury could lead to failure of axonal development and thus axonal degeneration. The remarkable exuberance of axonal growth during the premature period suggests a particular need for trophic support at this time. The consequences of axonal deficiency would be reduced cerebral and thalamic/basal ganglia volumes secondary to retrograde and anterograde effects such as projection fibres to and from cortex, the thalamus and the basal ganglia.

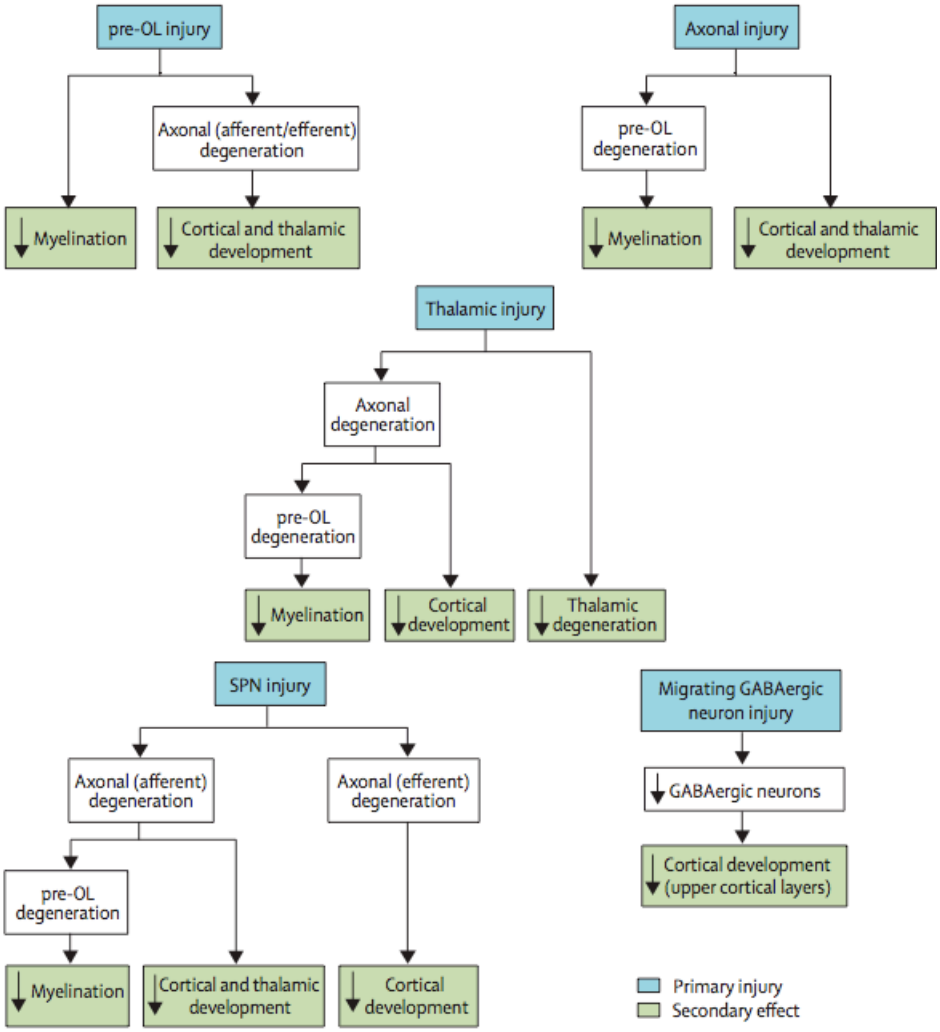


Fig. 1.11. Potential sequences of events leading to major brain sequelae observed with periventricular leukomalacia. Potential events are hypomyelination, and impaired cortical and thalamic development. for each sequence, the initiating primary injury is shown, and the subsequent secondary effects are postulated to occur because of maturational/trophic disturbances (Volpe 2009)

1.2.2.3. Axons

A recent neuropathological study revealed that diffuse axonal injury as determined by the apopototic marker fractin, occurs in the gliotic white matter in the acute and organizing stages of focal PVL (Haynes et al. 2008). Previously, axonal damage has been reported to occur within and at the periphery of periventricular necrotic foci of focal PVL, seen primarily as ß-APP immunopositive spheroids (Arai et al. 1995; Deguchi et al. 1997; Meng et al. 1997; Bell et al. 2005). In this study however, widespread axonal damage was found in regions where no ß-APP immunopositive spheroids were seen (Haynes et al. 2008). There was a correlation between the axonal damage and the chronological age of the periventricular focal necrosis reflecting the timing of the expression of the marker fractin, the extensiveness of

axonal damage and potential loss of axons (Haynes et al. 2008). Axonal damage is also suspected on diffusion tensor MRI studies (Huppi et al. 2001; Miller et al. 2002; Counsell et al. 2006; Anjari et al. 2007).

1.2.2.3.1. Thalami and basal ganglia

Recent imaging studies have described reduced thalamic and basal ganglia volumes in preterm infants at term equivalent age (Inder et al. 2005; Boardman et al. 2006; Woodward et al. 2006; Srinivasan et al. 2007). Reduced thalamic volumes were more marked in preterm infants with diffuse white matter injury (Boardman et al. 2006; Woodward et al. 2006). In a neuropathology study, Pierson et al showed that the incidence of gliosis and neuronal loss in the deep grey matter structures was significantly increased in PVL (Fig.1.12) (Pierson et al. 2007). Neuronal loss was absent in the group with diffuse white matter injury without necrosis in this study (Pierson et al. 2007). However in a subsequent study, neuronal loss, gliosis and axonal damage were noted in 59% with substantial involvement of the mediodorsal and reticular nuclei (Ligam et al. 2009). Haynes et al showed that approximately 31% of PVL cases had thalamic necrosis on histopathological examination (Haynes et al. 2008)

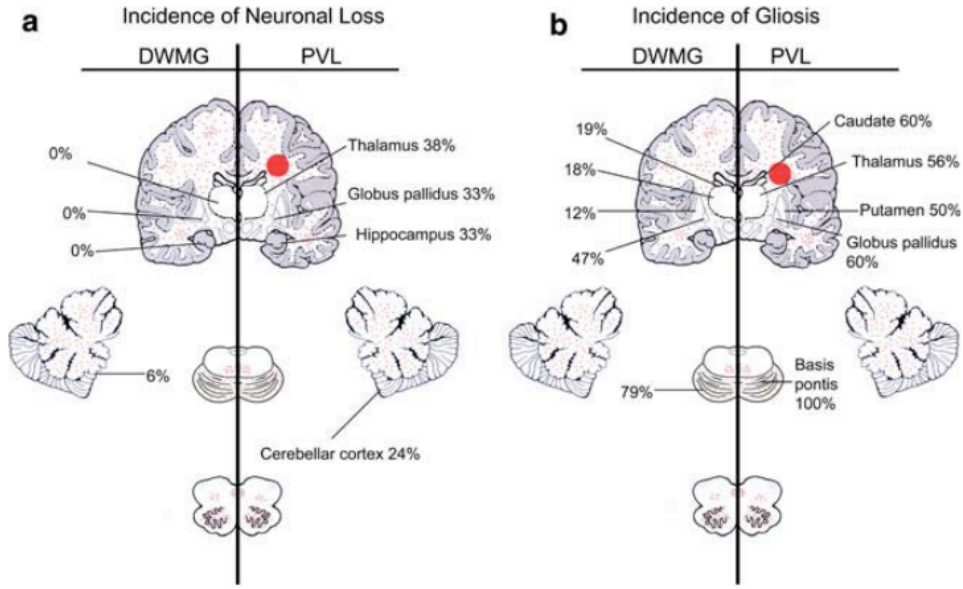


Fig. 1.12. Summary diagram comparing gray matter sites with a significantly higher incidence of a. neuronal loss, and b. gliosis in PVL and diffuse white matter gliosis (DWMG) cases. Gliosis of cerebral and cerebellar white matter, basis pontis, brainstem tegmentum and interior olives is depicted by small red dots, and focal, periventricular necrosis in the cerebral white matter (PVL) is denoted by large red periventricular circle (Pierson et al. 2007).

In imaging studies of preterm infants in childhood, reduced volumes of grey matter have been found (Peterson et al. 2000; Nosarti et al. 2002; Kesler et al. 2004; Reiss et al. 2004).

Hence, these findings could represent a direct injury to the grey matter and/or a maturational trophic disturbance such as disconnection of the thalamus from the developing cortex due to injury of the subplate neurons or impaired white matter tract development.

1.2.2.3.2. Cerebral cortex

MR imaging studies of preterm infants at term equivalent age have reported cortical abnormalities such as delayed cortical development, and decreased cortical volume (Peterson et al. 2000; Inder et al. 2005; Woodward et al. 2006). In children born preterm reduced cortical volumes were described with the most affected areas being the parieto-occipital, sensorimotor, premotor, temporal and hippocampal cortices (Isaacs et al. 2000; Peterson et al. 2000; Nosarti et al. 2002; Kesler et al. 2004; Kesler et al. 2008; Thompson et al. 2008).

1.2.2.3.3. Cerebellum

Cerebellar abnormality is common in preterm infants. In the neuropathological study by Pierson et al, neuronal loss in the dentate nucleus and the cerebellar cortex was found in 25-30% of the infants (Fig. 12) (Pierson et al. 2007). Gliosis was identified in the cerebellar cortex in 29% of the infants and in the basis pontis and inferior olive in 100% and 92% respectively. Reduced cerebellar volume has been found in preterm infants with supratentorial lesions such as haemorrhagic parenchymal infarction, intraventricular haemorrhage and PVL (Srinivasan et al. 2006) or diffuse white matter injury (Shah et al. 2006). In the absence of direct injury to the cerebellum such as cerebellar haemorrhage leading to cerebellar atrophy, the relationship of supratentorial brain lesions with cerebellar growth failure suggests that trophic interactions between cerebrum and cerebellum are present/disturbed.

1.3. MR Imaging

1.3.1. Conventional MR imaging and correlation with outcome

A broad range of abnormalities have been reported in qualitative studies of school-aged preterm children, including ventricular enlargement (Stewart et al. 1999), white matter damage (Olsen et al. 1997) and thinning of the corpus callosum (Cooke and Abernethy 1999; Stewart et al. 1999). Qualitative studies in preterm infants at term-equivalent age have reported cystic and diffuse white matter abnormalities, punctate

white matter lesions, cortical and deep grey matter abnormalities, ventricular dilatation, cerebellar haemorrhages, delayed myelination and thinning of the corpus callosum (Guit et al. 1990; de Vries et al. 1993; Barkovich and Sargent 1995; Leviton and Gilles 1996; Maalouf et al. 1999; Stewart et al. 1999; Sie et al. 2000; Counsell et al. 2003; Inder et al. 2003; Volpe 2003; Miller et al. 2005; Woodward et al. 2006; Limperopoulos et al. 2007; Leijser et al. 2009; Limperopoulos et al. 2009).

Conventional MR imaging at corrected age of term can give useful prognostic information when there is unilateral parenchymal involvement from PVL or from haemorrhagic parenchymal infarction that might present as asymmetrical myelination in the posterior limb of the internal capsule (PLIC) on conventional MR imaging at term equivalent. Asymmetrical myelination of the PLIC on MRI is predictive for subsequent development of a hemiparesis (De Vries et al. 1999; Roelants-van Rijn et al. 2001). However, its usefulness and accuracy for diagnosis and outcome prediction in diffuse white matter abnormality is still under investigation.

This is of increasing importance as the most common reported findings at term equivalent age are signal intensity abnormalities in the white matter, ventriculomegaly and thinning of the corpus callosum (Maalouf et al. 1998; Miller et al. 2005; Woodward et al. 2006). In a recent study early (within median 2 days after birth) MR imaging was normal in 44% of the preterm infants compared to 8% of preterm infants at term equivalent age (Dyet et al. 2006). The findings of early scanning are shown in Fig.1.13. Germinal matrix haemorrhage was the most common finding on early imaging whereas at term equivalent age diffuse excessive high signal intensity (DEHSI) in the white matter was the predominant finding (Fig.1.14)(Dyet et al. 2006).

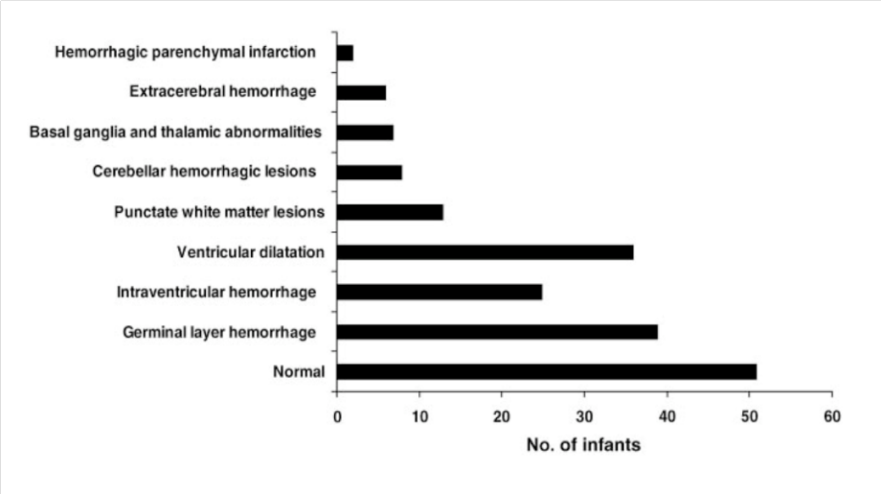


Fig.1.13. MR findings on the first MRI brain scans after birth (Dyet et al. 2006). Fifty-one (44%) infants had a normal initial MRI scan

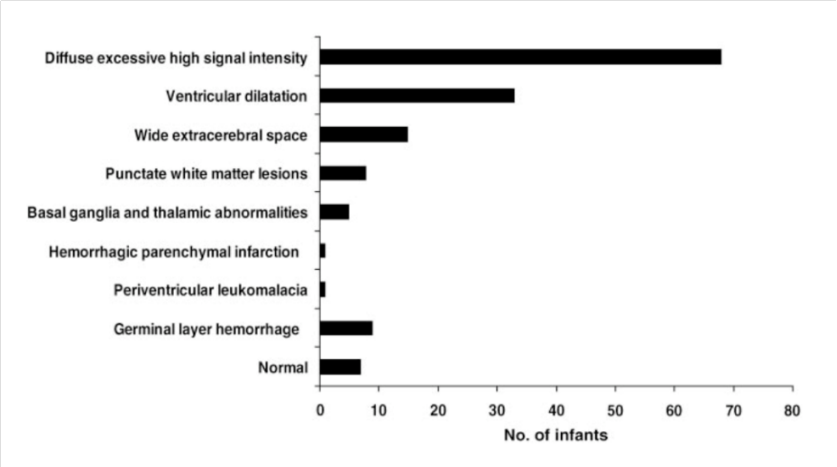


Fig.1.14. MR findings on the MRI scan at term equivalent age (Dyet et al. 2006). Only 8% of the term MRI scans were reported as normal.

Diffuse excessive high signal intenisty (DEHSI) on T2-weighted images was first described in 1999 by Maalouf et al in a MR study with serial scanning (Fig.1.15) (Maalouf et al. 1999). DEHSI was seen in the periventricular white matter and in the white matter of the centrum semiovale, and was found on the initial imaging in a few preterm infants of >28 weeks of gestation. On the imaging at term-equivalent age many more preterm infants showed DEHSI (Maalouf et al. 1999). At term-equivalent age, DEHSI was associated with ventricular dilatation and squaring of the ventricles. When infants with focal lesions were excluded from analysis there was a significant relationship between DEHSI and overall DQs at 18 months of age in a very small subgroup of preterm infants (Dyet et al. 2006).

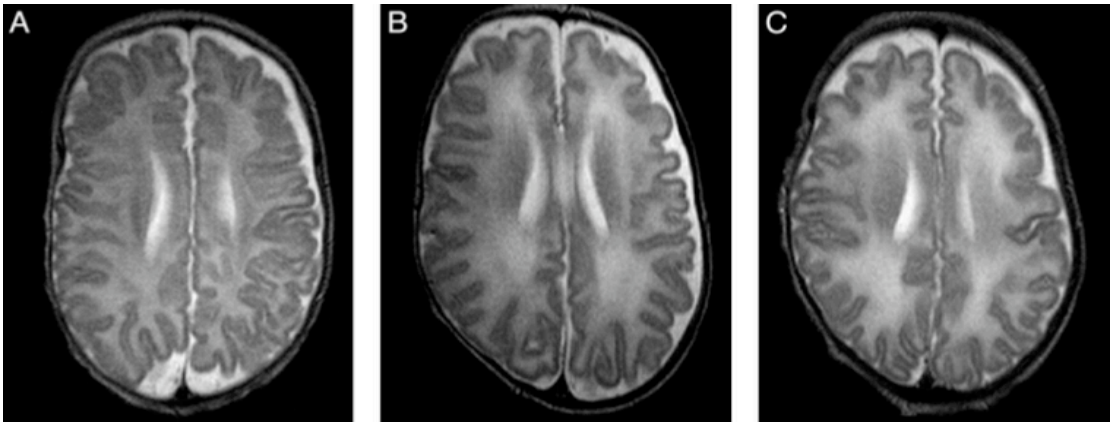


Fig.1.15. Axial T2 weighted images showing A. no DEHSI, B. mainly periventricular DEHSI and C. DEHSI extending into subcortical white matter (Dyet et al. 2006)

A large qualitative MR study on 167 preterm infants scanned at term-equivalent age has shown mild to severe WM abnormalities occurring in 72% of the preterm infants

and grey matter abnormalities in 49% (Woodward et al. 2006). 51% of infants had mild, 17% moderate and 4% severe white matter abnormalities; the severity of white matter abnormalities was highly correlated with the presence of grey matter abnormalities (Woodward et al. 2006). The applied scoring system of white matter abnormalities is based on five scales which assess the extent and nature of white matter signal abnormality, the loss in volume in the periventricular white matter, the extent of any cystic abnormalities, ventricular dilatation, and thinning of the corpus callosum (Fig.1.16). The scoring system for grey matter is based on three scales, which assess the signal intensity, gyral development and size of subarachnoidal space (Inder et al. 2003). Moderate to severe white matter injury were predictive for adverse cognitive, motor delay, cerebral palsy and neurosensory impairment at 2 years of age (Woodward et al. 2006). These qualitative MR scores were better predictors than any clinical or imaging measure, but there is still a deficiency in their sensitivity and specificity in predicting adverse outcome.

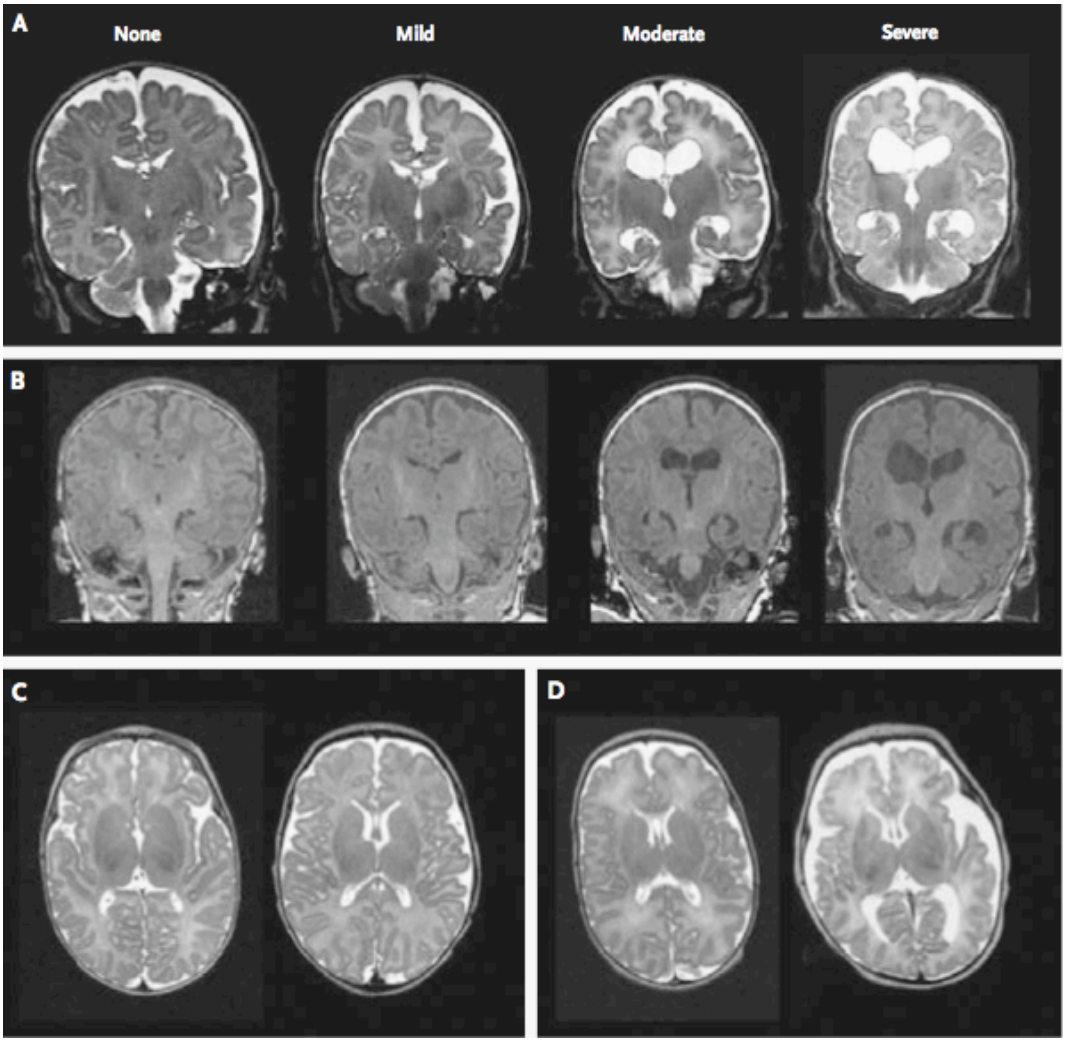


Fig.1.16. Representative MRI scans showing the increasing severity of white and grey matter injury of preterm infants included in the study (Woodward et al. 2006)

Miller et al classified the severity of white matter injury based on the findings of foci of abnormal T1-weighted hyperintensity in the absence of marked T2-weighted hypointensity in the white matter. White matter is classified as minimally abnormal if 3 or fewer areas of T1-weighted signal abnormality measuring <2mm are present, moderate if >3 areas of T1-weighted signal abnormality of <2mm present, but <5% of hemisphere, and severe if >5% of the hemisphere is involved (Fig.1.17) (Miller et al. 2005). His group found moderate/severe white matter abnormalities in 32% of the preterm infants (Miller et al. 2005). Abnormal outcome was associated with increasing severity of white matter injury, ventriculomegaly, and intraventricular haemorrhage (Miller et al. 2005).

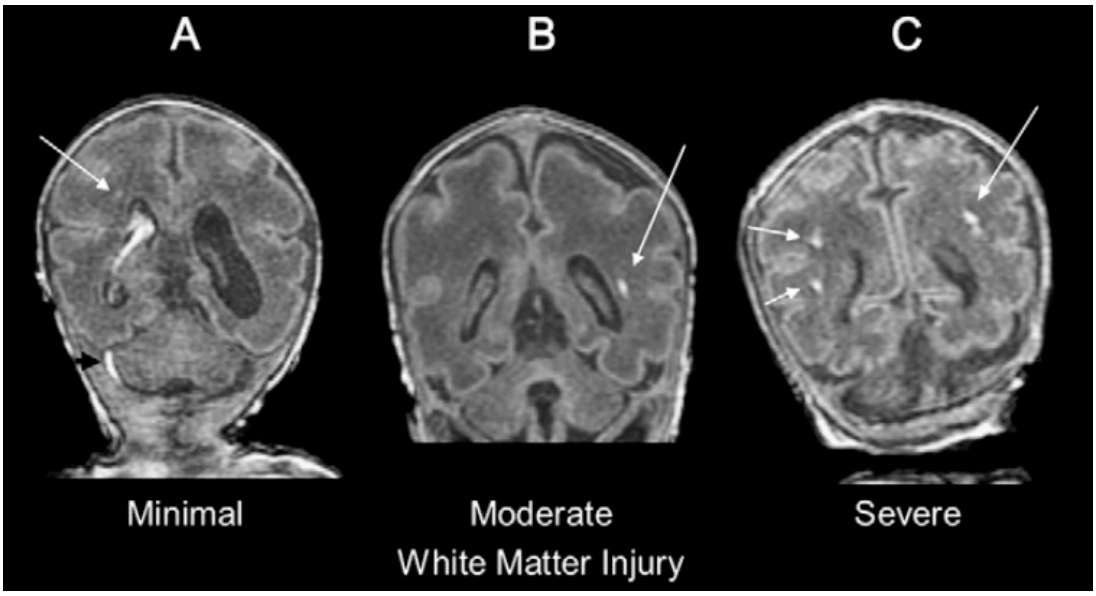


Fig.1.17. White matter scoring system showing foci of T1 weighted hyperintensities. A. mimimal, B. moderate and C. severe white matter abnormality (Miller et al. 2005)

However in other preterm MR studies, no correlation was found between such punctate white matter lesions and outcome (Fig.1.18) (Cornette et al. 2002; Dyet et al. 2006).

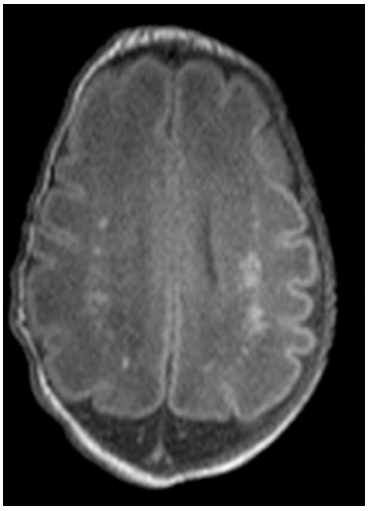


Fig.1.18. Punctate white matter lesions of preterm infant born at 29 weeks of gestation and scanned at 3 days of age (Dyet et al. 2006)

It is possible that there is a difference in the extent and location of white matter injury in the cohort of Miller compared to the cohorts of the other two studies in that extent of the punctate white matter lesions was much more severe in the American cohort. However, Ramenghi et al showed delayed maturation in preterm infants with punctate lesions in the white matter compared to preterm infants with normal white matter on imaging (Ramenghi et al. 2007). But until now, no follow-up study of this cohort has been published to establish the correlation of punctate white matter lesions with outcome.

By autopsy cerebellar haemorrhages are found in up to 10-25% of low birth weight infants (Grunnet and Shields 1976). Cerebellar haemorrhages were first described in ultrasound studies, occurring in about 3% of preterm infants (Merrill et al. 1998; Limperopoulos et al. 2005). The recent use of the mastoid fontanelle has improved the visualisation of the posterior fossa and hence the cerebellum (Di Salvo 2001). Limperopoulos et al. described cerebellar haemorrhages as being inversely related to birth weight; they report a greater degree of fetal-early-neonatal illness preceding the haemorrhage and a greater risk for subsequent neonatal morbidity and mortality (Limperopoulos et al. 2005).

In a preterm cohort studied with serial MRI scans, cerebellar haemorrhagic lesions were present in 7% of infants (Arthur 2006). Cerebellar lesions were related to low gestational age and birthweight and vaginal delivery, and were more common in those who died before follow-up. Most lesions were unilateral and half were associated with IVH. Cerebellar atrophy developed in two thirds (Dyet et al. 2006).

MRI has revealed non-haemorrhagic lesions of the cerebellum in preterm babies; some are cases of secondary atrophy probably resulting from infarction of the inferior cerebellar arteries (Johnsen et al. 2002; Bodensteiner and Johnsen 2005).

1.3.2. Quantitative MR imaging

1.3.2.1. T2 relaxometry

Quantitative MRI T2 relaxometry provides a promising opportunity for *in vivo* investigation and an objective quantitative measurement of tissue characteristics; T2 is a property of brain water protons in a selected volume of tissue at a given field strength. Relaxation times are time constants governing the process of returning to an equilibrium state for the protons (hydrogen nuclei) after radio-frequency pulse excitation (Tofts and du Boulay 1990). They characterize the average behaviour of an ensemble of nuclei interacting with their surroundings and are known to correlate with the water content and its dynamic structure in a biological system.

The major T2 determinant is the nature and frequency of interactions between water protons and tissue macromolecules; hence, T2 is influenced by the relative concentrations of tissue water molecules and the macromolecules with which they may interact (Bloembergen EM 1948) (Mathur-De Vre 1984). T2 reflects the strength of spin-spin exchange interactions between neighbouring spins. Higher spin density leads to stronger spin-spin exchange interactions thus shorter T2 times, and T2 in solids or highly viscous material is much shorter than in low-viscosity liquids. For water in a homogeneous environment, T2 relaxation can be expected to be mono-exponential. However, for water in an inhomogeneous environment such as brain, there are different T2 times corresponding to different water environments such as myelin bilayers, cytoplasm, and cerebrospinal fluid.

In vivo measurements of T2 distributions and water content in normal human adult brain revealed differences in brain tissue water environments not discernible on conventional MR images (Fig.1.19) (Whittall et al. 1997). The average of myelin water content of all white matter structures was significantly higher than that of all grey matter structures (Whittall et al. 1997).

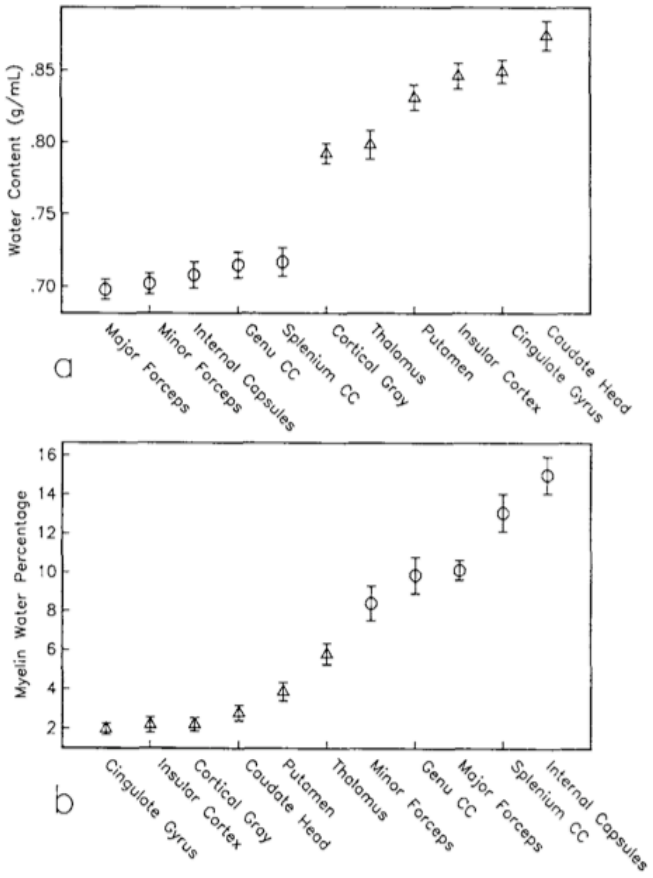


Fig.1.19. A. Mean water content in g/ml circles= white matter, triangles=grey matter. B. Mean myelin water percentage (Whittall et al. 1997)

The T2 relaxation decay of tissue water is most conveniently obtained from a multi-echo MR sequence in which the signal is refocused at multiple times during one acquisition.

1.3.2.1.1. T2 relaxometry in pathology

1.3.2.1.1.1. T2 relaxometry in neonatal encephalopathy

Positive correlations between prolonged thalamic and basal ganglia T2 and neurodevelopmental outcome severity in infants with neonatal encephalopathy have been described (Fig.1.20) (Shanmugalingam et al. 2006).

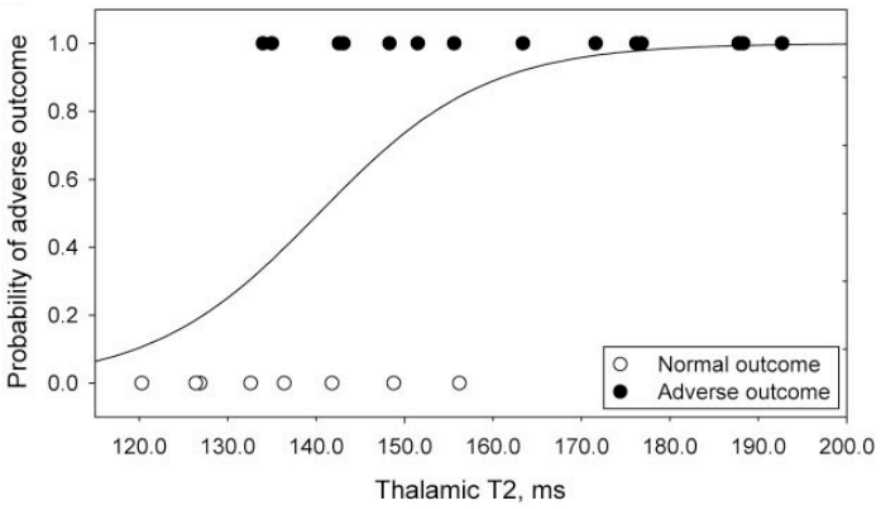


Fig.1.20. Predicted probability of an adverse outcome versus thalamic T2 (Shanmugalingam et al. 2006)

Although basal ganglia T2 was predictive (*p=0.049*) for outcome in infants after perinatal asphyxia (Boichot et al. 2006) (Shanmugalingam et al. 2006), in both studies MR spectroscopy more accurately predicted adverse outcome than T2.

1.3.2.1.1.2. T2 relaxometry in epilepsy

T2 relaxometry detects subtle brain abnormalities not seen on conventional imaging, as for example in epilepsy studies. The additional information is important in planning surgery and in predicting seizure burden after surgery. In one of the first T2 relaxometry studies of patients with partial epilepsy T2 quantitation improved the sensitivity of the MRI detection of hippocampal pathology (Jackson et al. 1993). Hence, T2 relaxometry enabled detection of mild and bilateral and progressive hippocampal abnormalities not recognized on visual assessment of the images (Jackson et al. 1993). Consistent with these initial findings, T2 relaxometry was described to be more sensitive than single-echo T2-weighted MRI (Coan et al. 2006). T2 relaxometry has also been shown to provide further information about the seizure focus in patients with temporal lobe epilepsy: temporal lobe white matter T2 values were prolonged in about 70% of patients with hippocampal atrophy and normal hippocampal volume (Townsend et al. 2004). T2 provided correct localisation of the seizure focus in 33% of patients with normal hippocampal volume, ie white matter T2 added useful lateralizing information compared to hippocampal T2 (Townsend et al. 2004). Furthermore, T2 abnormalities were found remote from the seizure focus (Scott et al. 2003; Briellmann et al. 2004). These studies involved manual placement of ROIs to then calculate T2 values. Recently, voxel-based morphometry (VBM), which enables the objective assessment of the whole brain, has been extended to

identification of T2 relaxation time abnormalities, termed voxel-based relaxometry (VBR), and its use has been demonstrated in patients with hippocampal sclerosis (Pell et al. 2004). More recently, multidimensional voxel-based analysis of GM-VBM, WM-VBM and VBR has been shown to improve information on structural changes associated with hippocampal sclerosis compared to an isolated analysis of a single modality (Pell et al. 2008). On neuropathological studies prolonged T2 in the hippocampus has been correlated with decreased neuronal cell counts in the dentate: T2 prolongation was mainly influenced by gliosis in the dentate gyrus (Briellmann et al. 2002). There has also been discussion as to whether temporal lobe white matter T2 is increased due to similar gliotic changes; the gliotic process in the white matter could be secondary to degeneration of white matter fibres connecting mesial structures to the rest of the brain through the temporal stem (Townsend et al. 2004).

1.3.2.1.1.3. T2 relaxometry in multiple sclerosis

In patients with multiple sclerosis, T2 relaxometry has shown promise in its ability to detect structural changes in normal appearing white matter that escape detection by conventional MRI; indeed, in a very recent study, it was shown that T2 can detect tissue damage in the global and regional cerebral normal-appearing white matter of multiple sclerosis patients that is missed by conventional lesion and atrophy measures (Neema et al. 2009). Furthermore, T2 relaxometry has also been useful in evaluating the short component of the T2-relaxation curve in normal-appearing white matter and overt lesions in patients with MS: the short T2 component is believed to reflect the myelin content (Laule et al. 2004). Reduced myelin detected by this technique has been reported in both types of lesion (Laule et al. 2004)

1.3.2.1.2. T2 relaxometry in brain maturation

Brain maturation is associated with T1 and T2 shortening (Fig.1.21) (Holland et al. 1986; Ferrie et al. 1999; Thornton et al. 1999), reduced water diffusion (Sakuma et al. 1991; Nomura et al. 1994; Wimberger et al. 1995; Neil et al. 1998; Miller et al. 2002), increased diffusion anisotropy (Toft et al. 1996; Huppi et al. 1998; Neil et al. 1998; Miller et al. 2002) and increased magnetisation transfer (Barkovich 2000).

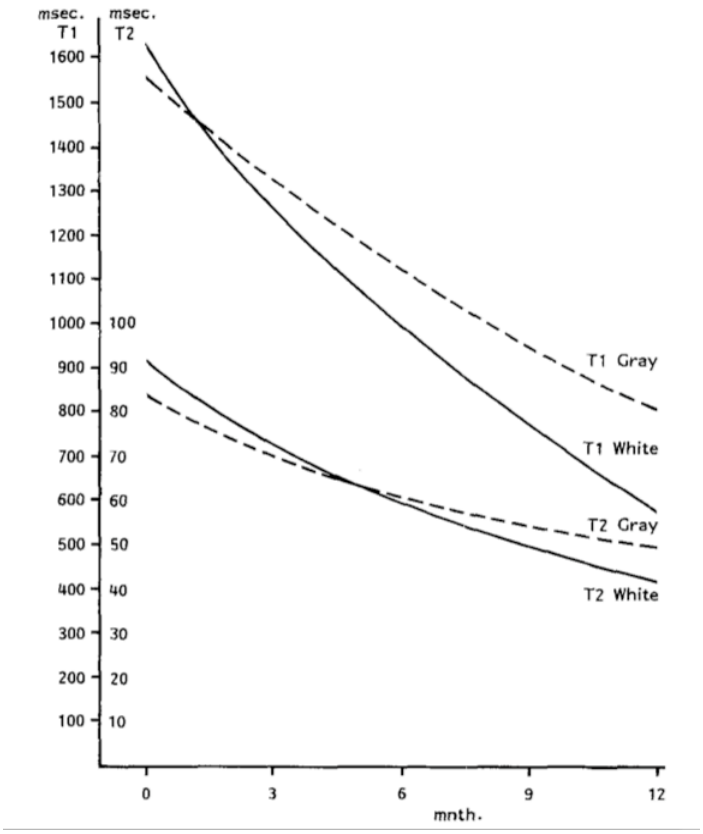


Fig.1.21. Relaxation values of grey and white matter by age (Holland et al. 1986)

These changes are thought to be largely the result of change in brain water content and pre-myelination and myelination. During brain maturation there is a marked decrease in brain tissue water content and increasing myelin-associated lipid and protein content (Fig.1.22. and Fig.1.23)(Dobbing and Sands 1973; Miot-Noirault et al. 1997; Van der Knaap 2005). Brain water content at birth is 88% and decreases to 82% by the age of six months (Dobbing and Sands 1973).

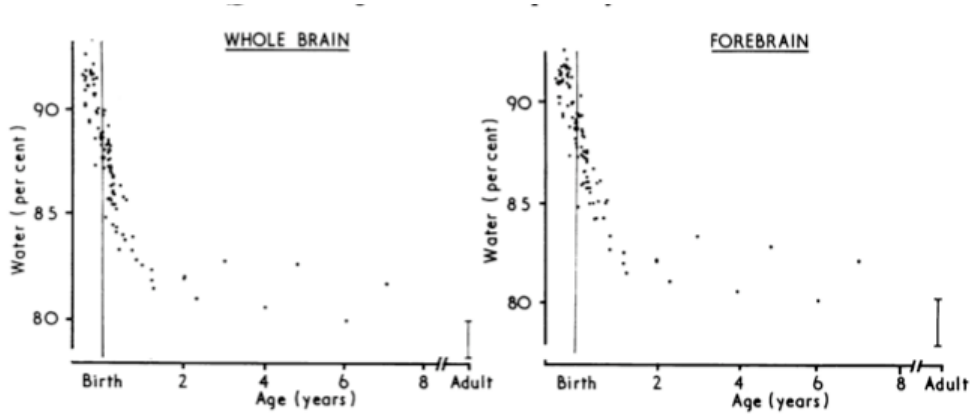


Fig.1.22. Percentage of water in whole brain and forebrain (Dobbing and Sands 1973)

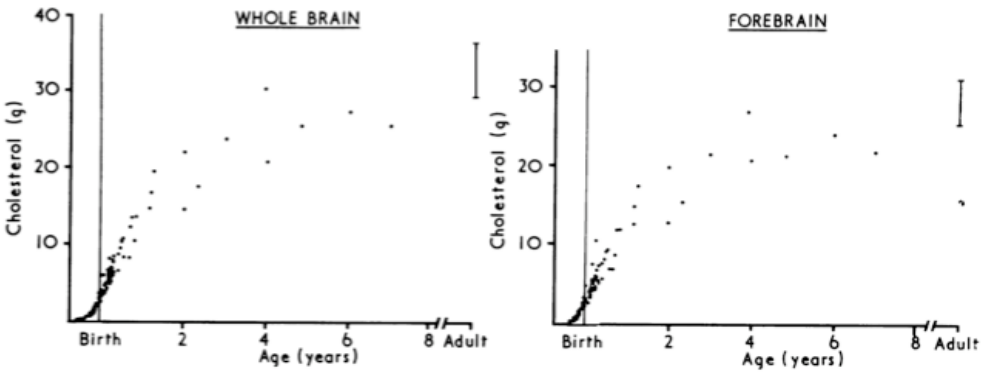


Fig.1.23. Concentration of cholesterol/unit fresh weight in whole brain and forebrain (Dobbing and Sands 1973)

In 1986 Holland et al measured T2 relaxation times in 13 brain regions of interest (ROIs) in 59 children ranged in age from 40 weeks to 16 years of age: T2 was higher than in adult brains and with increasing age a sharp decrease in T2, most pronounced in the first year of age, was seen (Holland et al. 1986). The infants were scanned at 0.35T and the reported T2 values at term were 91(SD 6) and 88 (SD 8) ms, respectively for white and grey matter.

Masumura et al studied children aged from two weeks to 15 years at 0.15T. The T2 range was 95-113ms at 44 weeks for regions incorporating white and grey matter (Masumura 1987). T2 was significantly prolonged in children aged 6 months compared to the adolescents; this prolongation was most pronounced in the white matter. Immediately after birth, T2 was markedly shorter in the corona radiata beneath the motor cortex than in other regions (Masumura 1987).

Baierl et al reported T2 relaxation times in 50 children between 3 months and 16 years of age confirming the findings of the early study by Holland et al (Baierl et al. 1988). T2 values were calculated from four regions of grey matter and seven regions of white matter. Field strength was 0.35T. Interestingly, grey matter T2 values were longer than white matter T2 (Fig.1.24)(Baierl et al. 1988).

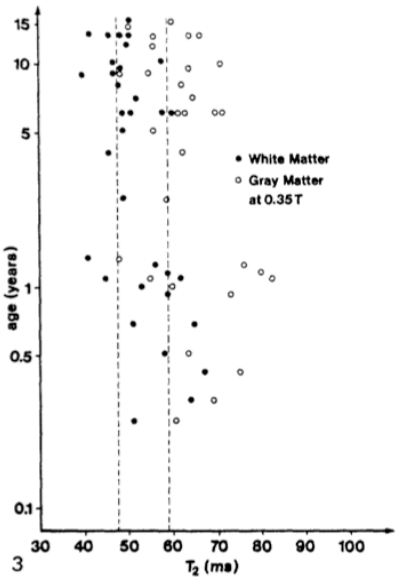


Fig.1.24. T2 relaxation times of grey and white matter as a function of time at 0.35T (Baierl et al. 1988)

In 1993, Ono et al reported on myelination by means of T2 in normal children and in children with known cerebral white matter diseases such as Pelizaeus-Merzbacher disease, dysmyelination and late Krabbe disease, using a 1.5T system. ROIs were drawn in the frontal and occipital deep white matter, centrum semiovale and in the corpus callosum; no grey matter regions were drawn. T2 values at 7 weeks of age were about 130ms and they reported the same pattern of T2 change over time with a sharp decrease within the first two years and reaching adult levels by 2 to 3 years of age (Fig.1.25) (Ono et al. 1993). The infants with white matter abnormalities showed prolongation of T2 (Ono et al. 1993).

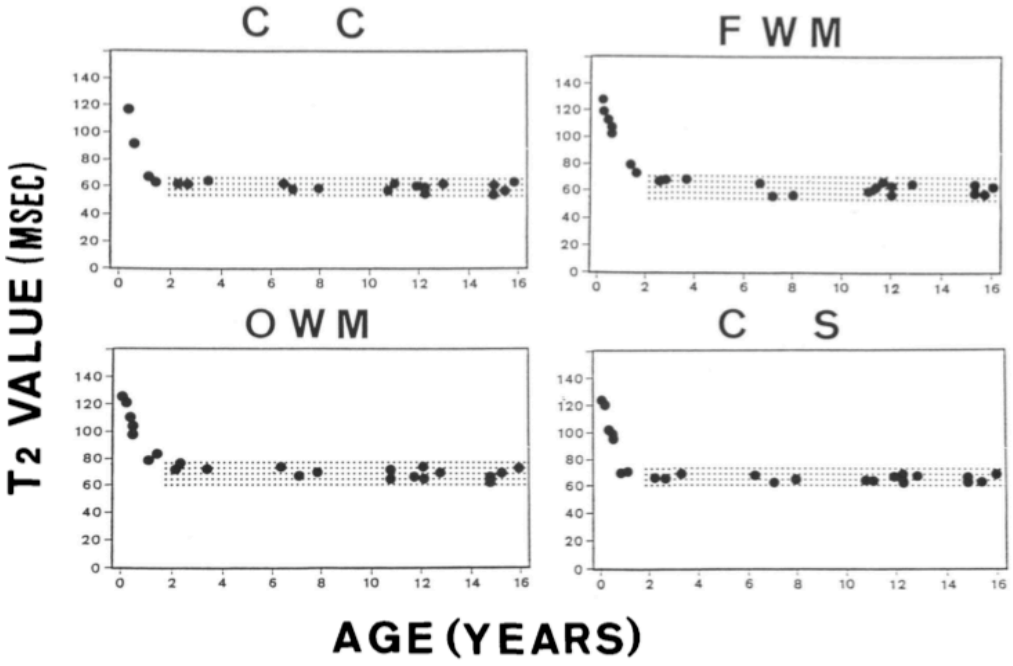


Fig.1.25. Changes in T2 with age. Shaded area indicates the mean SD (Ono et al. 1993)

In 1997, Miot et al reported on normal brain development in two animal models; three beagles were studied from birth to 4 months of age and nine baboons from birth to 30 months of age (Miot-Noirault et al. 1997). They determined the relaxation time profile for the normal myelination process in order to provide a reference for investigating pathologies. Their main findings were: i. at neonatal stages T2 values of both grey and white matter were higher in comparison to adults values; ii. T2 decreases with increasing age with a first period during which T2 decreases at a faster rate and a second period of gradual T2 decrease up to a stationary phase for which T2 were found to correspond to adult values; iii. decrease in T2 occurred from posterior to anterior (Fig.1.26). T2 kinetics measured within baboons brain were very similar to those observed in human brain (Miot-Noirault et al. 1997)

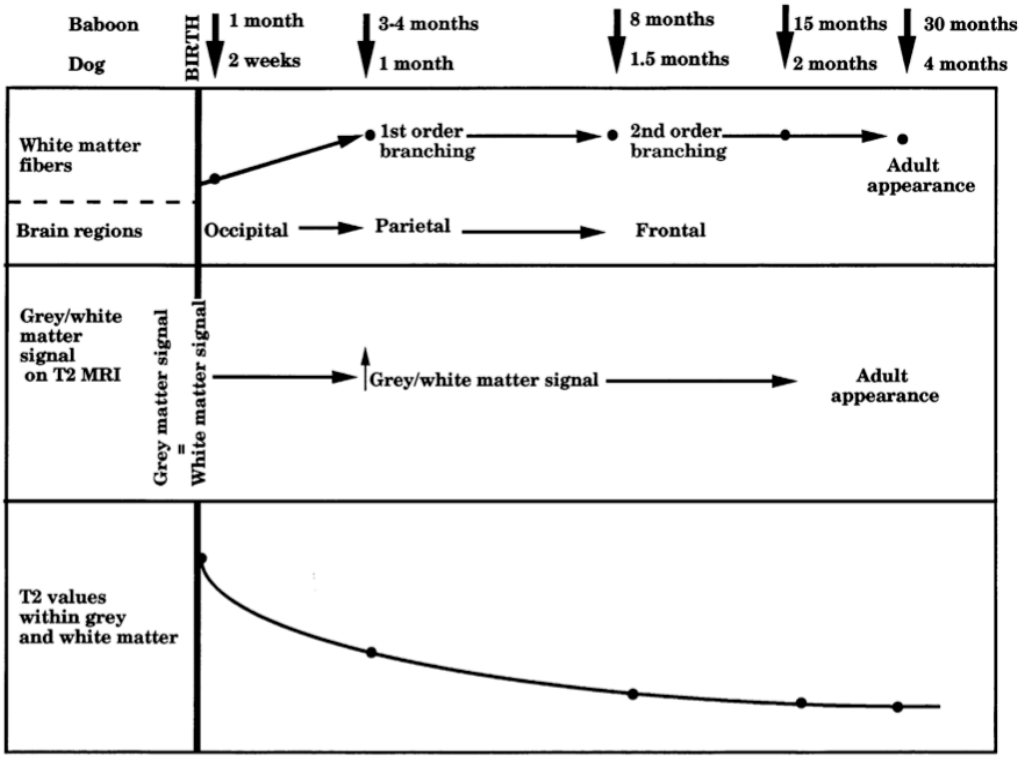


Fig.1.26. Diagramatic schema showing the cycle of myelination as observed on MR images and T2 kinetics analysis (Miot-Noirault et al. 1997)

As described by Ono et al, no significant difference between grey and white matter T2 for a given age could be observed (Miot-Noirault et al. 1997). Overall, these animal T2 findings were in keeping with the earlier T2 descriptions in paediatric brains.

However, in a kitten model, T2 was significantly longer in the white matter than in the grey matter of the newborn kitten brains whereas mature grey and white matter had similar T2 (Baratti et al. 1999). And again an earlier faster phase of T2 decrease with a later slower phase of T2 decrease was found (Fig.1.27)

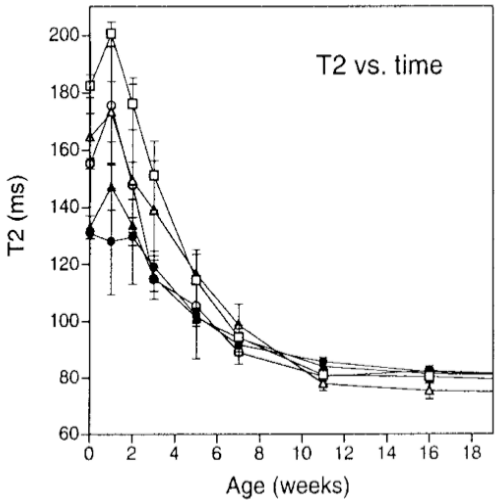


Fig.1.27.Time course for mean T2 in subcorticlal white matter, internal capsule, corpus callosum (Baratti et al. 1999)

Thornton et al reported on white and grey matter T2 in eleven healthy term infants with age range at scanning between 37 and 42 weeks (Thornton et al. 1999). The infants were scanned on a 2.4T system with TE=25,125,250, and 400ms. T2 was higher in white matter (periventricular white matter T2 (216.7ms (SD 33.3)) than in grey matter (thalamic T2 135.5ms (12.9)). A linear dependence of T2 upon gestational age was demonstrated in the thalami, periventricular and frontal white matter whereas no significant dependence of T2 upon gestational age was found in the parietal and pontine areas.

Fetal brain maturation was assessed by T2 relaxometry by Fulford et al (Fulford et al. 2001). An echo-planar imaging sequence was used with TE=74 and 484 ms and with 12 different echo values recorded. ROIs were drawn in the frontal and occipital white matter. A linear decrease in T2 with gestational age between 25 and 36 weeks was found.

Counsell et al scanned 18 preterm infants with median age at scanning of 31 weeks (range, 25-41 weeks), using a 1.0T system. These preterm infants were considered to be developing normally at one year of age and had normal conventional MR images (Counsell et al. 2003). A four echo pulse sequence was used with TE=30, 60,110, and 600ms. ROIs were drawn in the thalami, lentiform nuclei, frontal and occipital white matter, and central white matter at the level of centrum semiovale

(Counsell et al. 2003). A significant negative linear correlation between T2 and postmenstrual age was demonstrated in all white matter regions and the lentiform nuclei. Correlation between thalamic T2 and postmenstrual age just failed to reach significance (Counsell et al. 2003).

Ding et al described age-related T2 relaxation times in 70 healthy subjects aged between 3 weeks and 39 years (Ding et al. 2004). The imaging was performed on a 1.5T scanner with TE=14/71/128 or 15/75/135ms for children and 15/72/129ms for adults. Regions of interest were drawn in frontal and occipital white matter. Prolonged T2 values in newborn infants in comparison to the adults were found. With increasing age T2 decreased continuously, faster in the first two months, and slower thereafter. These data confirm the experimental kitten T2 data and human data published by Thornton et al in that white matter T2 values are significantly longer than grey matter T2 values during the neonatal period but almost the same in adult brains.

In a recent study looking at brain maturation beyond two years of age T2 was measured in the genu and splenium of the corpus callosum in 55 healthy subjects ages from 2 weeks to 35 years of age (1.5T scanner, TE=14, 71, and 128ms). Corpus callosum T2 decreased fast within the first months of life and very little after two years of age (Fig.1.28)(Ding et al. 2008).

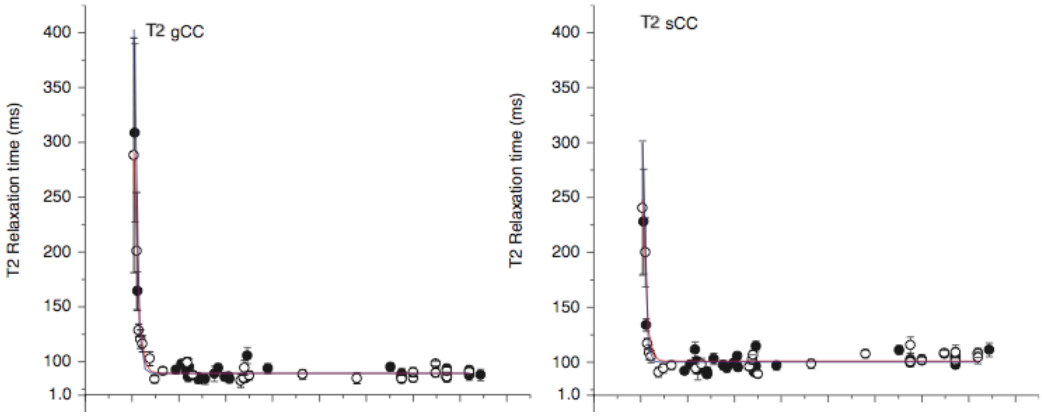


Fig.1.28. Numerical values and the corresponding curve fits of the T2 values obtained in the genu and splenium of the corpus callosum (Ding et al. 2008)

In summary, T2 appears to be a sensitive objective marker of brain maturation: it decreases during brain maturation, mainly due to the decrease in free water content, increase in concentration of myelin basic protein and proteolipid protein and increases in additional macromolecules. These processes cause more water protons

to interact with macromolecules leading to a decrease in T2, although contrast changes on T2 weighted images may not be evident.

1.3.2.1.3. T2 relaxometry in preterm infants

Ferrie et al published the first quantitative T2 study in preterm infants, looking at T2 in different brain regions. Seven preterm infants with mean gestational age of 30 weeks were scanned at 37 weeks corrected age (Ferrie et al. 1999). These infants had normal qualitatively assessed MR images and normal neurodevelopmental assessment at one year of age. This study did not include normal controls for T2 comparison. MR scans were performed on a 2.35T system, and T2 relaxometry data were acquired using TE= 60, 90,120, and 150ms. ROIs were drawn within white and grey matter on T2 maps. They found highly prolonged T2 values in comparison to adults. Mean T2 increased from 213±28 ms in occipital white matter, to 227±21 ms in parietal white matter, to 234±27 ms in temporal white matter and to 266±35 ms in frontal white matter. In frontal and occipital regions, T2 values rose from periventricular to the subcortical white matter (Ferrie et al. 1999). The conclusion of their findings was that T2 variations might be due to pre-myelination and could indicate different stages of brain maturation.

Abernethy et al scanned 103 preterm infants at age seven years on a 0.5T system (Abernethy et al. 2003). Four ROIs were drawn in the white matter: right and left hemispheric white matter and in the right and left hippocampus. In children without visible brain lesions on conventional imaging, central white matter T2 was significantly increased in preterm infants with minor motor impairments. This is the only T2 relaxometry study that correlates white matter T2 with outcome in preterm infants; note though that the imaging was done at seven years of age.

Williams et al published the first T2 relaxation study at 3T in neonatal brain. They studied 13 infants with suspected neurological abnormalities: two term infants and 11 preterm infants (4 preterm infants were scanned at term equivalent age, two had intraventricular haemorrhage and two PVL) (Williams et al. 2005). TEs were 30, 60, 100, 160, 200, and 250ms. They also analysed the relaxation rates R1 and R2 (R1=1/T1, R2=1/T2). ROIs were drawn in the frontal, posterior, periventricular white matter, frontal and posterior grey matter at the level of the centrum semiovale, and in the basal ganglia and thalamus. They found interregional variation of R2 and R1. R2 seemed to provide better discrimination between grey and white matter. R2 values were similar to those reported at lower field strength (Williams et al. 2005).

A recent imaging study included 12 very preterm infants with gestational age ranging from 25 to 29 weeks, 11 preterm infants with gestational age ranging from 29 to 32 weeks and 10 normal term infants (Rose et al. 2008). All infants were scanned at around 41 weeks corrected age. The imaging protocol included diffusion tensor imaging and T2 relaxometry with TE of 26/128/192ms. ROIs were drawn corresponding to white matter regions with significantly altered fractional anisotropy (FA) (tract-based spatial statistics and ROIs) measured between preterm and control infant groups. Significantly increased T2 was found in the very preterm group compared to the preterm group in anatomical locations corresponding to altered FA (Fig.1.29). These ROIs were within the sagittal striatum, frontal lobe white matter, external capsule, commissural tracts within the corona radiata and the white matter at the level of the centrum semiovale (Rose et al. 2008). Significantly increased T2 was found in the corticospinal tract within the cerebral peduncles in term infants compared to preterm infants; no other regions with significantly altered T2 were found when preterm were compared with term infants (Rose et al. 2008).

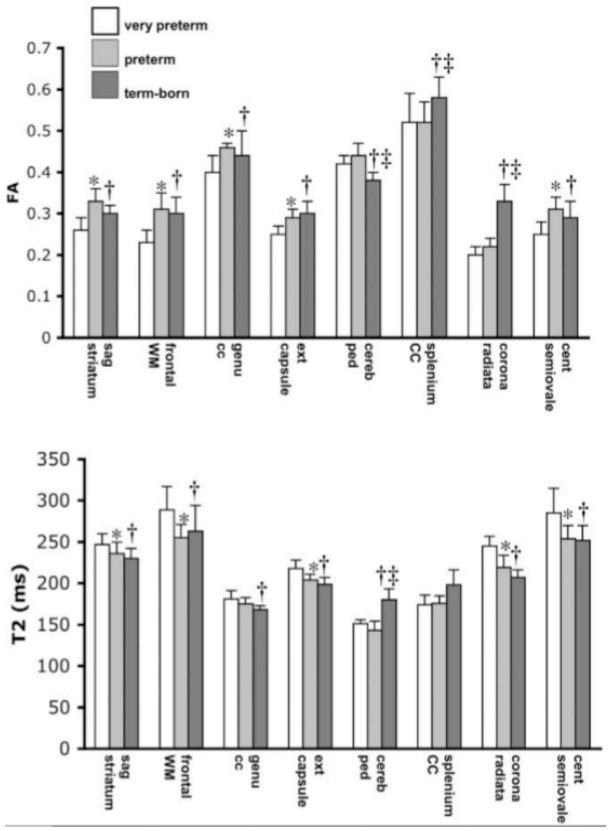


Fig.1.29. Representative bar graphs showing the distribution of MRI values for the anatomical regions of interest with significantly different FA and T2 values across the three groups. ∗Significant differences between very preterm and preterm,†very preterm and term born, and ‡preterm and control (Rose et al. 2008)

1.3.2.2. Diffusion weighted and tensor imaging

Diffusion refers to Brownian molecular motion and can be quantified by calculating the apparent diffusion coefficient (ADC). Diffusion weighted imaging (DWI) measures the diffusivity/displacement of water molecules. The measurement of water diffusion in the human brain is of great clinical interest because it provides a sensitive and early indicator of brain injury. ADC decreases rapidly after acute CNS injury. For example, in adult patients who suffered a stroke, conventional imaging techniques do not show any evidence of injury for several hours or even days after the insult (Yuh et al. 1991) whereas DWI reveals the injury within minutes (Warach et al. 1996; Bydder et al. 2001). Similar findings are reported in neonatal stroke (Bydder et al. 2001). In PVL, diffusion weighted images were able to detect white matter abnormalities before conventional images were abnormal (Inder et al. 1999; Bozzao et al. 2003; Fu et al. 2009). Such early detection and quantitative assessment of brain injury is critical in the context of early neuroprotective interventions.

Water diffusion may also be evaluated and expressed in forms other than a simple average across all directions. Diffusion anisotropy is the condition in which apparent diffusion coefficients are not the same when measured along different axes of a sample (Neil 1997); this is due to barriers to water motion that are more restrictive in one direction than another. In the brain for example, motion of water is less hindered when movement is parallel to axons than it is when movement is perpendicular to axons. As a result, average diffusion parallel to myelinated axons is greater than diffusion perpendicular to them (Neil 1997). In the cerebrospinal fluid water motion is isotropic, i.e. equivalent in all directions. In white matter however, water diffuses in a highly directional, or anisotropic, manner (Fig.1.30).

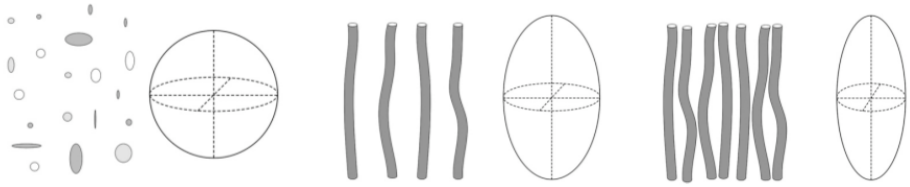


Fig.1.30. Isotropic diffusion with spherical ellipsoid. In white matter, fibers are oriented in bundles, diffusion is anisotropic, the anisotropy increases with fiber density and decreasing membrane permeability(Huppi and Dubois 2006).

Diffusion tensor imaging (DTI) describes the preferential direction of water diffusivity, and yields three diffusion tensor eigenvalues (λ_1, λ_2, λ_3), which represent the diffusion along the three principal axes. In general, DTI data are used to calculate two basic properties: the overall amount of diffusion (the sum of the three eigenvalues = ($\lambda_1 + \lambda_2 + \lambda_3$)) and the directionality (anisotropy) of diffusion. The

primary eigenvalue λ_1 measures the diffusion along the axons and is called axial diffusivity. Higher values reflect better axonal integrity. The secondary λ_2 and tertiary λ_3 eigenvalues measure the diffusion perpendicular to the axons; they are often averaged ($[\lambda_2+\lambda_3]/2$) to produce a measure of radial diffusivity. This provides a measure of the degree of restriction of diffusion perpendicular to the axons. FA and relative anisotropy (RA) are indicators of the degree of water diffusion anisotropy independent of the overall water diffusion coefficient. Both parameters are zero for isotropic diffusion and increase up to $\sqrt{2}$ and 1 for RA and FA respectively. If high diffusion levels and low anisotropy are seen in white matter, then it is indicative of poorly developed, immature or injured white matter. High levels of anisotropy are considered a reflection of coherently bundled, myelinated fibers along the axis of greatest diffusion.

1.3.2.2.1. ADC and FA in the developing brain

In 1991, Sakuma et al described white matter ADC in neonatal brain to be significantly higher than in infant (<5 months) and adult brain (Fig.1.31) (Sakuma et al. 1991). T1 and T2-weighted images obtained concurrently showed no evidence of development of myelination in the corresponding areas. This finding led to the concept of earlier detection of neonatal brain development with diffusion weighted imaging than with conventional imaging (Sakuma et al. 1991). Nomura et al described increasing anisotropy in healthy full term infants up to six months (Nomura et al. 1994).

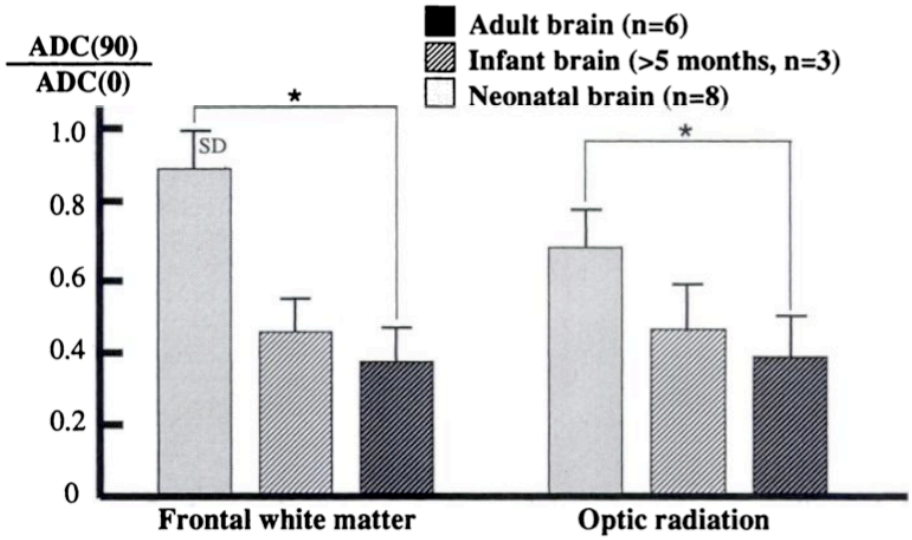


Fig.1.31. Comparison of average ratio of diffusional anisotropy in frontal white matter and optic radiation among neonates, infants and adults (Sakuma et al. 1991)

In a study on ADC and anisotropy in normal brain in newborn infants, higher ADC values were found in neonatal than adult brains confirming earlier publications (Neil et al. 1998). Furthermore, statistically significant correlations between average diffusion and gestational age were found in all brain regions analysed: average diffusion decreased with increasing gestational age (Fig.1.32) (Neil et al. 1998). This correlation was present for grey and white matter. In contrast to those findings, anisotropic values did not show a statistically significant correlation with gestational age in most regions, except for the white matter of the centrum semiovale and the white matter in the occipital lobes (Fig.1.33). The idea that the decrease of average diffusion is due to a reduction of water content is supported by the finding of no associated increase of anisotropy with increasing gestational age. If the change in hindrance of water is proportional along all axes then there will be no change in anisotropy (Neil et al. 1998). Anisotropy values in the white matter of the centrum semiovale seemed to increase more rapidly near the end of gestation rather than early in gestation (Fig.1.33).

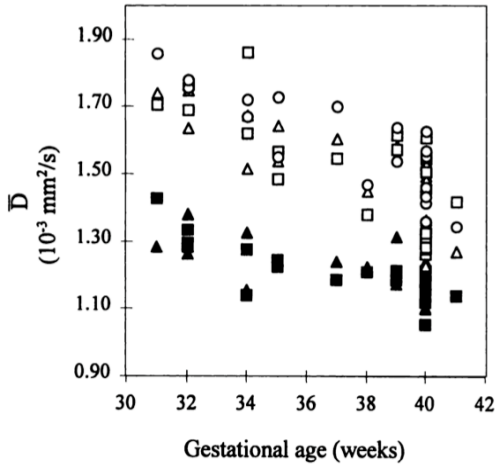


Fig.1.32 Plot of average diffusion in various brain regions versus gestational age (Neil et al. 1998)

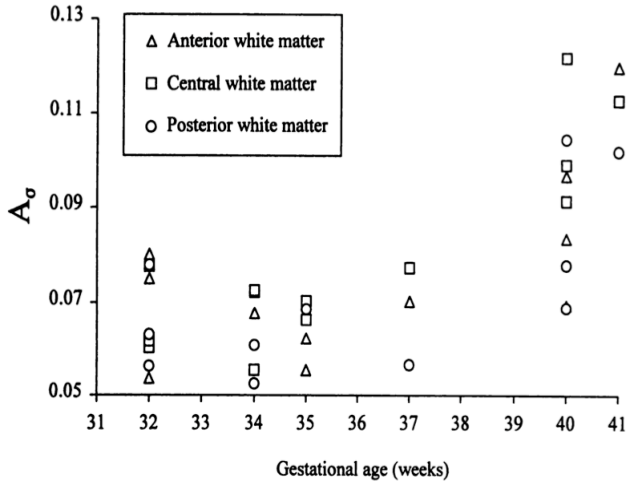


Fig.1.33 Plot of anisotropy in white matter of centrum semiovale versus gestational age (Neil et al. 1998)

The white matter of the centrum semiovale near term is in a premyelinating state, a state which is associated with an increase in axonal diameter, axonal membrane changes and an increase in the concentration of microtubule-associated proteins (Wimberger et al. 1995). This could explain the sharp increase in anisotropy near term age in that region, well before visible changes on T1- and T2-weighted images (Barkovich et al. 1988).

Mukherjee et al found three distinct patterns of maturational changes in anisotropy with increasing age (115 subjects age ranging from 1 day to 11 years): a smaller increase in the grey matter of the basal ganglia, a large nonlinear increase in white matter structures, and an intermediate pattern in the thalamus (Mukherjee et al. 2001). The age-dependent rise of anisotropy in the white matter paralleled the decline in ADC.

Forbes et al showed a logarithmic decline of ADC within the first year of life (Forbes et al. 2002). The correlation of ADC with age was stronger in the white matter than in the grey matter. ADC varied widely with brain location with the most marked differences found in the white matter; lowest ADC values were found in the posterior limb of the internal capsule and sequentially higher values in the anterior limb of the internal capsule and then subcortical white matter corresponding to the recognised pattern of myelination. This was an important study contributing to the description of regional differences of ADC in the brain.

Other studies have described increased anisotropy with age in specific white matter structures (Boujraf et al. 2002; McGraw et al. 2002; Gilmore et al. 2004; Schneider et al. 2004; Provenzale et al. 2007; Saksena et al. 2008). Strong positive correlations between anisotropy measures in major white matter tract and age throughout childhood into adolescence have been reported (Schmithorst et al. 2002; Schneider et al. 2004; Barnea-Goraly et al. 2005; Ben Bashat et al. 2005; Snook et al. 2005; Provenzale et al. 2007; Saksena et al. 2008). Likewise, studies demonstrated negative correlations between overall diffusion and age (Mukherjee et al. 2002; Schmithorst et al. 2002; Snook et al. 2005; Zhang et al. 2005). Cerebellar white matter development in children was also assessed by MD and FA (Saksena et al. 2008).

Recently, Gilmore et al described early postnatal development of the corpus callosum and corticospinal tracts in 47 newborn infants with a mean gestational age

at birth of 39.8 ±0.9 weeks and age at scanning of 43.1 ±1.7 weeks and 42.6 ±1.6 weeks for girls and boys respectively (Gilmore et al. 2007). T1-weighted and T2-weighted signal intensity, mean diffusivity (MD) and fractional anisotropy were measured in the central of splenium and genu of the corpus callosum (CC), and the corticospinal tract. MD was significantly different across all ROIs studied in genu, splenium, and left corticospinal tract; the central region of the corticospinal tract had the lowest MD. FA was significantly different across all studied ROIs in genu, splenium, and left corticospinal tract (Fig.1.34). Although the age range was narrow, age-related changes in FA and MD in fibers of genu and corticospinal tract were detected. T2-weighted and T1-weighted signal intensities were different across all white matter tracts. Whereas there was no change in T1-weighted signal intensity with age, T2-weighted signal intensity declined significantly with age in all regions except for the central part of the splenium (Fig.1.35) (Gilmore et al. 2007). Hence, the central regions of white matter tracts appear more mature and organised than the peripheral cortical regions, with lower MA and higher FA. In this study, T2-weighted signal appeared to be more sensitive to maturational events in white matter than T1-weighted signal intensity. This study described age-related regional differences in MD and FA in the neonatal period.

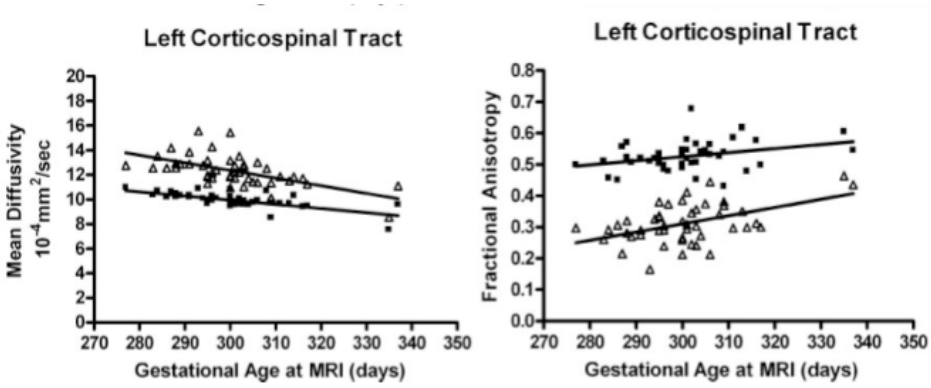


Fig.1.34. Significant correlation between mean diffusivity/FA in the left corticospinal tract and gestational age on MRI.

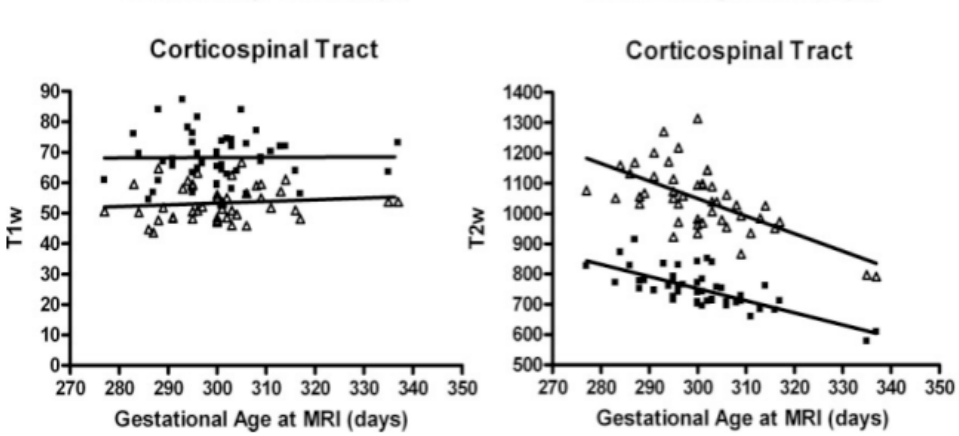


Fig.1.35. Whereas no significant decline with age was noted in T1-weighted signal intensity, T2-weighted signal intensity decreases with age r^2=0.181, p=0.025 (Gilmore et al. 2007)

In a cohort of normal children aged between 5-18 years, significant sex-age interactions on FA and MD were shown; differing developmental trajectories in white matter between boys and girls were observed (Schmithorst et al. 2008). Males had higher anisotropy in associative white matter regions, whereas females had higher anisotropy in the splenium. Females had lower ADC in the corticospinal tract and frontal white matter of the right hemisphere, while males had lower ADC in the most superior aspect of the corticospinal tract of the right hemisphere and in the occipito-parietal regions (Schmithorst et al. 2008).

Most recently, maturational changes of ADC_{av}, RA, FA and eigenvalues in 73 patients with age range of 3 weeks to 19 years were described. The findings were consistent with previous published work: diffusion in all directions declined with age with most pronounced changes in λ_2 and $\lambda_{3,}$ especially for regions with high anisotropy like corpus callosum, pons and internal capsule (Lobel et al. 2009). Changes for ADC_{av} closely reflect changes observed for the three eigenvalues. RA and FA increased with age, but the age influence was different for RA than for FA. Interestingly within a brain region, different diffusion parameters did not necessarily reflect the same amount of changes (Lobel et al. 2009). Hence, it seems important for microstructural assessment of brain regions to calculate all diffusion parameters to understand the mechanisms underlying changes in brain tissue.

In summary, the increase in normal white matter anisotropy with age appears to take place in three steps (Dubois et al. 2008). First, progressive fiber organisation in fascicles is likely to leave water content, membrane density and thus mean diffusivity relatively unchanged, but may lead to an increase in anisotropy, due to increased longitudinal diffusivity and decreased transverse diffusivity (Fig. 1.36)(Dubois et al. 2008).

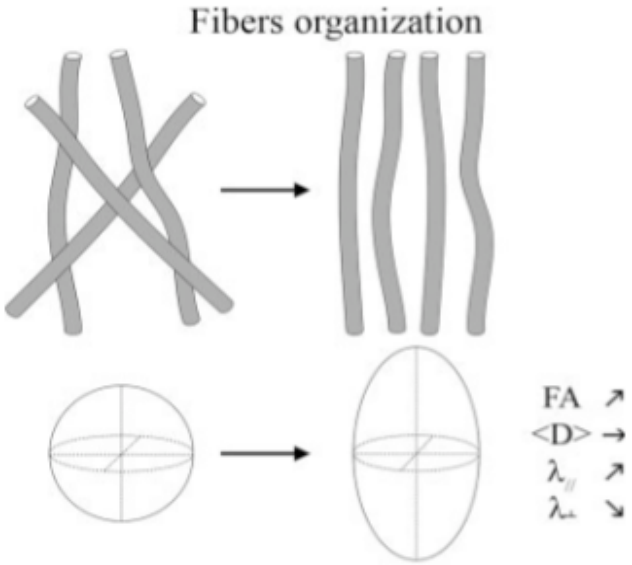


Fig.1.36. First step in maturational processes and diffusion indices in the white matter (Dubois et al. 2008).

The second step takes places before the histological appearance of myelin, the premyelinating state. This state is characterised by an increase in the number of microtubule-associated proteins in axons, a change in axon calibre, and a significant increase in the number of oligodendrocytes, with a decrease in water content (Wimberger et al. 1995; Prayer et al. 2001). As this process is rather isotropic, it should lead to a decrease in the three diffusivity indices, without significant decrease in anisotropy (Fig.1.37)(Dubois et al. 2008).

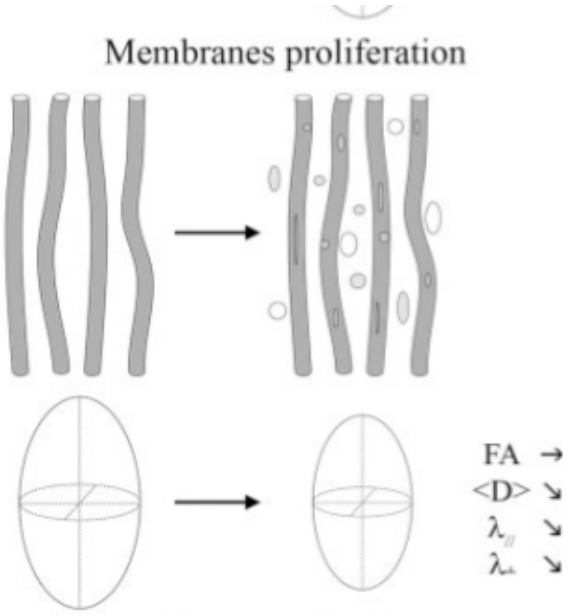


Fig. 1.37. Second step in maturational processes and diffusion indices in the white matter (Dubois et al. 2008)

Third, the last phase of “true” fibers myelination, corresponding to the ensheathment of oligodendroglial processes around the axons. The anisotropy should increase while mean diffusivity should decrease due to unchanged longitudinal diffusivity contrasting with decreased transverse diffusivity (Fig.1.38) (Dubois et al. 2008).

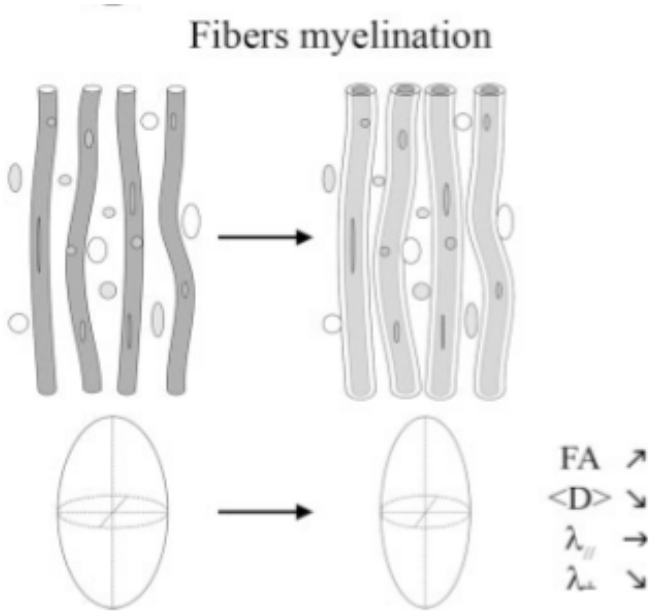


Fig.1.38. Third step of maturation in white matter (Dubois et al. 2008)

1.3.2.2.2. ADC and FA in the developing brain of preterm infants with or without white matter injury

In 1998, Hüppi et al reported on the assessment of white matter in 17 preterm infants and 7 full term infants by diffusion tensor imaging. Preterm infants at term-equivalent age showed higher mean ADC in the central white matter and lower relative anisotropy in the posterior limb of the internal capsule and central white matter compared with full term infants (Fig.1.39) Full term infants and preterm infants at term showed marked differences in white matter fiber organisation as shown by vector maps (Huppi et al. 1998).

Diffusion measure	Preterm at term (n = 10)	Fullterm (n = 7)	p value
Apparent Diffusion Coefficient (μm^2/ms ± SD)			
White matter	1.4 ± 0.2	1.2 ± 0.1	0.016
Internal capsule	1.1 ± 0.01	1.1 ± 0.01	0.5
RA (% ± SD)			
White matter	10.9 ± 0.6	22.9 ± 3.0	0.001
Internal capsule	24.0 ± 4.5	33.1 ± 0.6	0.006

Fig.1.39. Comparison of ADC and RA in central white matter and internal capsule in preterm infants at term and full term infants (Huppi et al. 1998)

In a subsequent study twenty preterm infants with gestational age of 29±1.9 weeks were studied with conventional MRI within the first 3 days of life and at term-equivalent age, with diffusion tensor imaging in addition at term age. Preterm infants were grouped with or without white matter injury, which was characterised by diffuse or nodular periventricular T1-weighted hypointensities or T2-weighted hyperintensities. No differences in white matter ADC were found among preterm infants with or without white matter injury. By contrast, relative anisotropy in the posterior limb of the internal capsule was lower in the white matter in preterm infants with white matter injury than in those without (Fig.1.40) (Huppi et al. 2001).

Diffusion Measure	WM Regions				
	Internal Capsule	Central WM	Frontal WM	Occipital WM	Temporal WM
ADC (μm^2/ms ± SD)					
No WM injury	1.0 ± 0.1	1.5 ± 0.2	1.7 ± 0.1	1.6 ± 0.2	1.4 ± 0.1
WM injury	1.1 ± 0.1	1.5 ± 0.2	1.7 ± 0.1	1.5 ± 0.2	1.2 ± 0.5
P value	NS	NS	NS	NS	NS
RA (% ± SD)					
No WM injury	22.8 ± 4.7	12.9 ± 3.3	8.2 ± 1.4	9.1 ± 1.0	13.9 ± 1.8
WM injury	17.2 ± 3.9	9.5 ± 1.7	9.0 ± 1.4	7.7 ± 1.8	11.9 ± 2.2
P value	.02	.03	.3	.06	.7

Fig.1.40. Comparison of ADC and RA in different white matter regions in preterm infants with and without white matter injury (Huppi et al. 2001)

In a case report published by *Inder et al*, diffusion weighted images showed abnormalities (decreased ADC) in cerebral white matter at a time when neither cranial ultrasound nor conventional MRI detected any abnormality. These DWI abnormalities later acquired classical MR imaging features of cystic PVL (Inder et al. 1999). Similar findings were subsequently published (Bozzao et al. 2003; Fu et al. 2009).

Serial quantitative diffusion tensor imaging was performed in preterm infants with and without white matter injury (Miller et al. 2002). ADC was substantially higher in white matter than in grey matter. In preterm infants with or without minimal white matter injury, white matter ADC significantly decreased with increasing age. However, in preterm infants with moderate white matter injury ADC did not change with increasing age in the posterior white matter and a significant increase was seen in the frontal white matter and visual association areas. Similarly, anisotropy increased linearly with increasing age in preterm infants with normal white matter whereas in infants with moderate white matter injury, anisotropy did not increase with increasing age in any white matter regions except for the corticospinal tract. The changes in anisotropy in infants with white matter injury were more prominent than the changes in ADC (Miller et al. 2002). The failure of ADC to decrease and anisotropy to increase

normally in preterm infants with white matter injury may be an early marker of brain injury in the developing brain. This study also showed that there might be a selective vulnerability of certain white matter regions. No discussion of comparison of ADC and RA values of both preterm groups at term equivalent age was given but since an absence of decrease of ADC and increase of RA is described in preterm infants with moderate white matter injury, one can presume that RA and ADC might be significant differently at term age between preterm infants with and without white matter injury.

In a large study of 50 preterm infants conventional MR and diffusion weighted imaging was performed at term-equivalent age. The preterm infants were divided into three groups based on their MR findings: a. normal white matter, b. DEHSI, or c. overt white matter lesions. ADC values in frontal, central and posterior white matter of the centrum semiovale were significantly higher in the preterm group with DEHSI and in infants with overt white matter lesions than in those with normal white matter (Fig.1.41) (Counsell et al. 2003). No significant ADC differences between preterm infants with DEHSI and those with overt white matter lesions could be found. In preterm infants with normal white matter, no regional ADC differences were found in the analysed white matter regions. For infants with DEHSI, there was no significant difference in ADC between frontal and posterior and between central and posterior white matter. However, ADC in the frontal white matter was significantly higher than in the central white matter in this group.

Region	ADC Value (Mean [± SD]) × 10^{-3} mm^2/s		
	Normal White Matter	DEHSI	Overt White Matter Pathology
Frontal white matter	1.361 ± 0.10	1.565 ± 0.16	1.605 ± 0.12
Central white matter	1.287 ± 0.12	1.429 ± 0.14	1.502 ± 0.15
Posterior white matter	1.315 ± 0.11	1.535 ± 0.14	1.572 ± 0.10

Fig.1.41. Mean ADC values for the three groups (Counsell et al. 2003)

This was an interesting finding and in contrast to a previous published study by Hüppi et al where no differences in ADC between preterm infants with normal and abnormal white matter was found (Huppi et al. 2001). One could speculate that the reason for those discrepancies lies within the definition of white matter injury defined by conventional MR imaging by both groups. Whereas Hüppi et al defined white matter injury by the presence of punctate lesions within the white matter, Counsell et al grouped the infants according to the presence of DEHSI. The absence of punctate

lesions does not imply that there is no DEHSI (Dyet et al. 2006); hence, some of the "normal white matter" infants in the study by Hüppi et al *could* have had DEHSI and vice versa. It should also be noted that in the initial study by Hüppi et al, higher ADC values were reported in preterm infants compared to term controls, a finding which is consistent with the studies by Counsell et al (Huppi et al. 1998; Counsell et al. 2003; Counsell et al. 2006).

The initial findings by Counsell et al were confirmed by a subsequent study in 2006 (Counsell et al. 2006). This study included term controls. ADC was significantly increased in preterm infants with DEHSI compared to term controls and preterm infants with normal appearing white matter. In addition to increased ADC increased radial and axial diffusivity was shown in white matter of the centrum semiovale, frontal, periventricular and occipital white matter in preterm infants with DEHSI compared to preterm infants with normal-appearing white matter and term controls. These findings led the authors to suggest that DEHSI is a neuroimaging correlate of white matter injury and might represent widespread axonal and oligodendrocyte abnormalities. Higher white matter ADC values at the level of the centrum semiovale were negatively correlated with developmental quotient at corrected two years of age (Krishnan et al. 2007). Higher ADC and lower FA were also found in preterm infants with white matter abnormalities on conventional MR at term equivalent age in a Swedish preterm population (Skiold et al.). In contrast to those studies, are the findings by Hart et al showing no differences in ADC in preterm infants without DEHSI compared to those with DEHSI (Hart et al. 2011).

Fourteen preterm infants without evidence of white matter abnormalities by conventional MRI were studied with DTI (Partridge et al. 2004). The aim was to quantify tract-specific characterisation of maturing white matter. Significant differences in DTI parameters were observed between white matter pathways with earlier maturing commissural fibers of the corpus callosum and deep projection tracts of the cerebellar peduncle and internal capsule exhibiting lower mean diffusivity and high FA than later maturing subcortical projection and association pathways. It was shown that maturational changes in white matter tracts, including reduction of mean diffusivity and increases in fractional anisotropy with age, were primarily due to decreases in two diffusion eigenvalues (λ_2 and λ_3) (Fig.1.42) (Partridge et al. 2004).

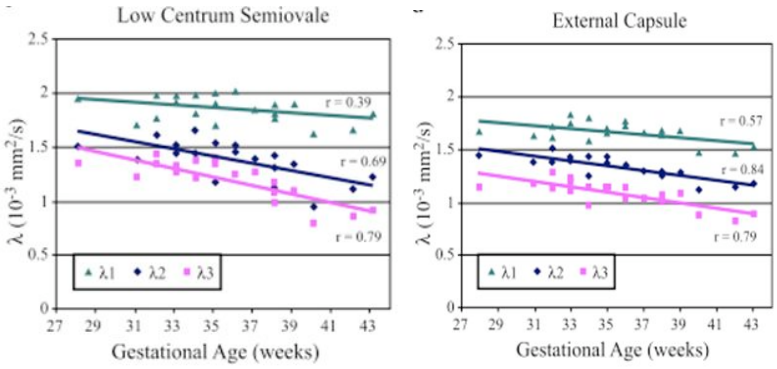


Fig.1.42. Comparison of changes of the three eigenvalues in. the white matter of the centrum semiovale and the external capsule. Most dramatic changes were observed in the minor eigenvalues λ_2 and λ_3 (Partridge et al. 2004).

These findings were similar to those in previous work. The new observation was that the reduction in mean diffusivity and increase in anisotropy is primarily due to reduction in diffusion eigenvalues λ_2 and λ_3 (Partridge et al. 2004). This is in keeping with studies of brain maturation into childhood where the greatest decreases are in λ_2 and λ_3 with comparatively little change in λ_1 (Mukherjee et al. 2002). The increase of anisotropy caused by reduction of diffusion eigenvalues λ_2 and λ_3 suggests that changes in anisotropy are not only explained by the decrease of brain water content, but more likely reflect microstructural changes of premyelination and myelination causing increased hindrance to water diffusion perpendicular to the direction of axonal fibers.

Berman et al performed DTI to examine the motor and sensorimotor white matter tracts in preterm infants between 28 and 43 weeks of gestational age. FA, mean diffusivity and λ_1, and transverse diffusion (mean of λ_2 and λ_3) in both the motor and sensory pathways were significantly correlated with age: increasing anisotropy and decreasing mean diffusivity with age (Berman et al. 2005). Tractography was used to derive quantitative DTI measures. Consistent with previous published work transverse diffusivity decreased with age at a significantly higher rate than the primary eigenvalue. DTI parameters were significantly higher in the sensory pathway than in the motor pathway (Berman et al. 2005).

Another DTI study found a statistically significant correlation between gestational age and FA in the PLIC and differences of FA were observed between the white matter tracts (Dudink et al. 2007)

Tract-based spatial statistics (TBSS) is an automated observer-independent approach for assessing groupwise microstructural differences in the major white pathways of the brain. It has been recently applied in the preterm brain and showed that regions within the centrum semiovale, frontal white matter and genu of the corpus callosum had significantly lower FA in preterm infants scanned at term-equivalent age compared to term controls (Anjari et al. 2007). Preterm infants born below 28 weeks of gestation displayed additional reductions in FA in the posterior aspect of the posterior limb of the internal capsule, the external capsule and the isthmus and middle portion of the body of the corpus callosum and had larger areas of reduced anisotropy in the white matter of the centrum semiovale, frontal white matter and genu of the corpus callosum (Anjari et al. 2007).

One hundred and eleven preterm infants were scanned at term-equivalent age. A white matter signal intensity score was applied to group the infants. ADC, FA and axial and radial diffusivity were measured in the posterior limb of the internal capsule, frontal, sensorimotor, and occipital white matter (Cheong et al. 2009). Infants with extensive white matter signal intensity abnormalities had significantly lower FA in the posterior limb of the internal capsule, right inferior frontal regions and right superior occipital regions. ADC was higher in bilateral sensorimotor regions and right superior occipital regions. A region-specific altered diffusivity was noted involving particularly the sensorimotor pathways (Cheong et al. 2009). These findings are in keeping with previous published worked by the Hammersmith group (Counsell et al. 2003).

A diffusion anisotropy study of the cerebral cortex of preterm infants ranging from 26 to 41 weeks of gestation showed maximal anisotropy of water in the cortex at 26 weeks and anisotropic values approaching zero by 36 weeks of gestation; this was presumed to reflect a radial organisation of the cerebral cortex that was present at 26 weeks but disappears by 36 weeks (Fig.1.43) (McKinstry et al. 2002).

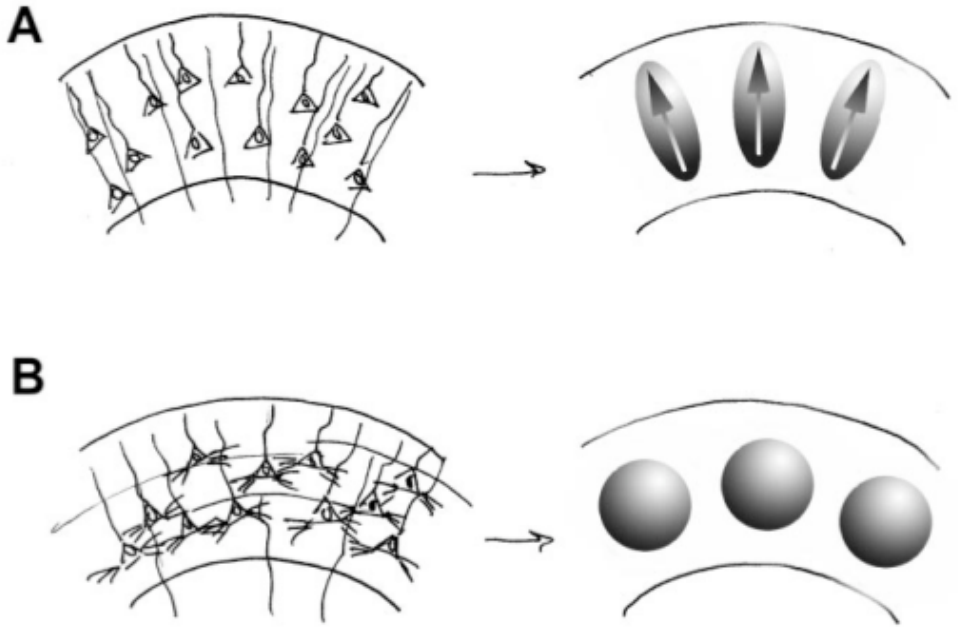


Fig.1.43. A diagram depicting an explanation for the cortical anisotropy. A. at 26 weeks, radial glial fibers and pyramidal neurons with prominent radially oriented apical dendrites. B. By 35 weeks, prominent basal dendrites for the pyramidal cells and thalamocortical afferents have been added with the effect of restricting water displacement uniformly in all directions resulting in diffusion ellipsoids that are spherical (McKinstry et al. 2002).

Cerebral cortex during preterm maturation was assessed using DTI, compared to the macrostructural development of cortical gyration evaluated using three-dimensional volumetric T1-weighted MR images (Deipolyi et al. 2005). 37 preterm infants with gestational age ranging between 25 to 38 were scanned at a mean age of 32.8 weeks (range 25.3 to 38.1 weeks). Nine preterm infants had normal white matter; all others had white matter scores between 1 and 3 according to the score published by Miller et al (Miller et al. 2002). MD, FA and the three eigenvalues were measured in the same cortical regions. Cortical gyration, FA, and radial diffusivity (λ_1) were significantly inversely correlated only with increasing age (Deipolyi et al. 2005). The correlation with radial diffusivity was an interesting finding as it is exactly the reverse pattern of eigenvalue changes that have been observed in the maturation of white matter in newborn infants and children (Mukherjee et al. 2002; Partridge et al. 2004). The authors suggested that the major eigenvalue λ_1 corresponds to the magnitude radial to the pial surface. Thus, the relatively large decline in λ_1 with increasing age is mainly due to a reduction in the radial component of diffusivity (Deipolyi et al. 2005), which is consistent with previous published work (McKinstry et al. 2002; Gupta et al. 2005).

Both in cerebellar grey and white matter ADC decreased and FA increased with increasing age in preterm infants. Severe intraventricular haemorrhage was associated with increased ADC in the middle cerebellar peduncles, hila of the cerebellar nuclei and cerebellar cortex, and decreased FA in all these regions (Tam et al. 2009).

1.3.2.2.3. Correlation between ADC/FA and outcome in preterm infants

1.3.2.2.3.1. Motor outcome

Arzoummanian et al found significant lower FA at term equivalent age in the right posterior limb of the internal capsule and in the cerebral white matter in preterm infants with abnormal neurological outcome (other than cerebral palsy) compared to preterm infants with normal motor development (Arzoumanian et al. 2003). No significant differences in ADC were found between the groups but there was a trend toward higher ADC in the cerebral white matter in preterm infants with abnormal neurological outcome.

In a serial diffusion tensor imaging study 24 preterm infants were categorised into a control group and groups with mild and severe brain injury. The first scan was performed at 10-14 days of life and the second around 36 weeks corrected gestational age (Drobyshevsky et al. 2007). 12 infants had neurodevelopmental testing at 2 years of age. ADC was higher in the severe injury group in the central and occipital white matter and the corona radiata. FA was lower in the optic radiation compared to controls. A significant correlation was found between a low psychomotor scale index at 2 years of age and low FA in the posterior limb of the internal capsule found; this correlation was not significant on the second scan.

FA in the posterior limb correlated with the severity of gait deficits at 4 years of age. Higher GMFCS values in preterm infants with low neonatal FA and the strong correlation found between neonatal FA and GMFCS at 4 years suggest that neonatal FA in the posterior limb of the internal capsule may predict severity of motor deficits that develop later in childhood (Rose et al. 2007). In a subsequent study by the same group, 78 preterm infants scanned at term equivalent age had neurodevelopmental assessment at 18 months of age (Bayley Scale of Infant Development). Children with abnormal neurodevelopmental outcome at 2 years had more abnormalities on conventional MR images and reduced splenium and right posterior limb FA (Rose et al. 2009). Abnormal neurodevelopment was more common in males than in females.

Males had more MR abnormalities and lower FA and higher ADC in the splenium and right posterior limb of the internal capsule (Rose et al. 2009).

Lower FA values in parts of the corpus callosum were found using TBSS in preterm infants at term equivalent age and they were linearly correlated with DQ at two years corrected age (Counsell et al. 2008).

1.3.2.2.3.2. Cognitive and behavioural outcome

Nagy et al found in neurological normal children aged between 8 and 18 years a positive correlation between working memory capacity and FA in the superior and inferior left frontal lobe whereas the reading ability only correlated with maturation of white matter in the left temporal lobe (Nagy et al. 2004). This study showed that maturation shown by anisotropy affects cognitive functions. Further studies showed positive correlation between FA and temporo-parietal white matter (Beaulieu et al. 2005; Deutsch et al. 2005; Niogi and McCandliss 2006). Schmithorst et al reported that anisotropy measures in frontal and occipito-parietal association areas were related to full-scale IQ in school-age children (Schmithorst et al. 2005).

Neurologically normal preterm infants scanned between 8.8 and 11.5 years showing minimal changes on conventional MR images had reduced white matter anisotropy and white matter volume which correlated significantly with full-scale IQ (Fig.1.44) (Yung et al. 2007).

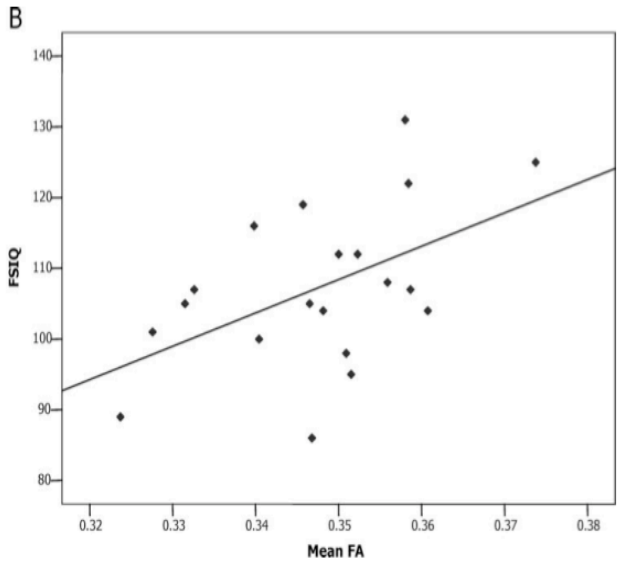


Fig.1.44. Scatterplot showing mean FA of white matter versus full-scale IQ (FSIQ) at 8 years of age; r^2=0.497, p=0.005 (Yung et al. 2007)

Abnormal high MD in the genu of the corpus callosum was associated with lower performance IQ in female adolescent preterm infants (Kontis et al. 2009).The association between IQ and corpus callosum abnormalities is consistent with a recent study showing that neuropsychological performance relates positively to callosal FA and negatively to MD and that typical cognitive development is supported by white matter maturation, as measured by DTI (Fryer et al. 2008). The brain structure-function relationships observed in that study suggest that optimal cognitive functioning in adolescents is correlated with a high degree of white matter coherence (Fryer et al. 2008).

Nagy et al reported that 11 years old preterm infants with diagnosed attention deficits had lower anisotropy in the posterior corpus callosum and internal capsule than sex-matched control infants (Fig.1.45) (Nagy et al. 2003); these findings were similar to a study in children and adolescents with ADHD demonstrating decreased anisotropy in frontal, cerebellar, and left parieto-occipital areas (Ashtari et al. 2005). ADHD children and adolescents had significantly lower FA in the corticospinal tract and the superior longitudinal fasciculus compared with controls (Hamilton et al. 2008).

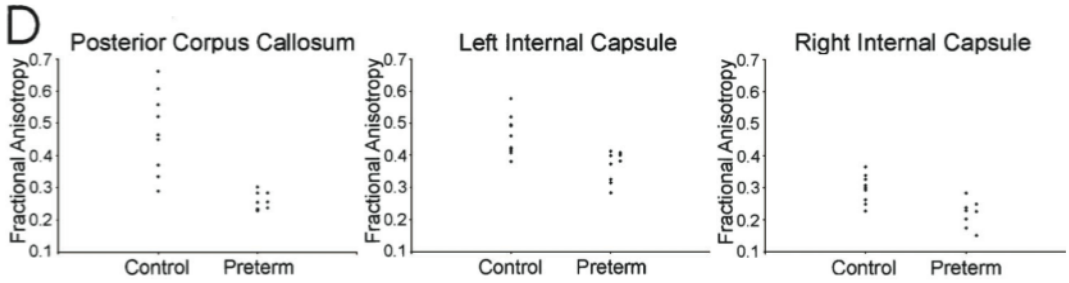


Fig.1.45. Plots of FA values from the peak of the three brain regions. Values of preterm with PVL are lightly to the right (Nagy et al. 2003).

Many DTI studies in adolescents born preterm show that anisotropy in white matter structures is altered and correlates with later cognitive and motor outcome (Eikenes et al. ; Nagy et al. 2003; Vangberg et al. 2006; Skranes et al. 2007; Constable et al. 2008; Kontis et al. 2009).

However, no preterm study relating ADC/FA measured at term-equivalent age to later cognitive outcome was found.

A recent study evaluated the visual function in preterm infants at term-equivalent age and found a direct relation between development of the white matter in the optic radiation assessed by TBSS and visual function (Bassi et al. 2008).

1.4. Neurodevelopmental outcome in preterm infants

1.4.1. Motor outcome

1.4.1.1. Cerebral Palsy (CP)

CP prevalence is around 2 per 1000 live births and increases to 40-100 per 1000 live births among infants born very early or with low birth weight (Himmelmann et al. 2005). In the European CP study, the majority of children with CP were born at term age; however, among children with CP 45.5% were preterm infants: of these, 24% were born below 28 weeks of gestation, 35% were between 28 and 32 weeks and 41% were born between 32 and 36 weeks of gestation. CP is the major disabling motor outcome following preterm birth, which is diagnosed in about 5% of very low birth weight infants (VLBW) (Surman et al. 2003; Platt et al. 2007). In extremely low birth weight infants (ELBW) the rate is reported as high as 8-20% (Wood et al. 2000; Hack et al. 2005; Marlow et al. 2005; Robertson et al. 2007; Larroque et al. 2008). Spastic CP is the most commonly reported type of CP among preterm infants. During the 1990s increased survival of extremely preterm infants appeared to be associated with an increased morbidity (Winter et al. 2002; Wilson-Costello et al. 2005). However, recent reports show a significant fall in the prevalence of CP among VLBW infants from 60.6 per 1000 live births in 1980 to 39.5 per 1000 live births in 1996 (Platt et al. 2007). Interestingly, the significant decline in prevalence was restricted to the group of preterm infants with birth weights of 1000-1499g, hence, it was mainly explained by a reduction of the prevalence of bilateral spastic CP amongst infants born at 28-32 weeks or weighing 1000-1499g (Fig.1.46) (Platt et al. 2007). Using gestational age revealed similar findings, for infants born between 28 and 31 weeks the prevalence fell from 80 to 50 per 1000 live births whereas the prevalence for infants aged <28 weeks remained static at 40 per 1000 live births (Platt et al. 2007).

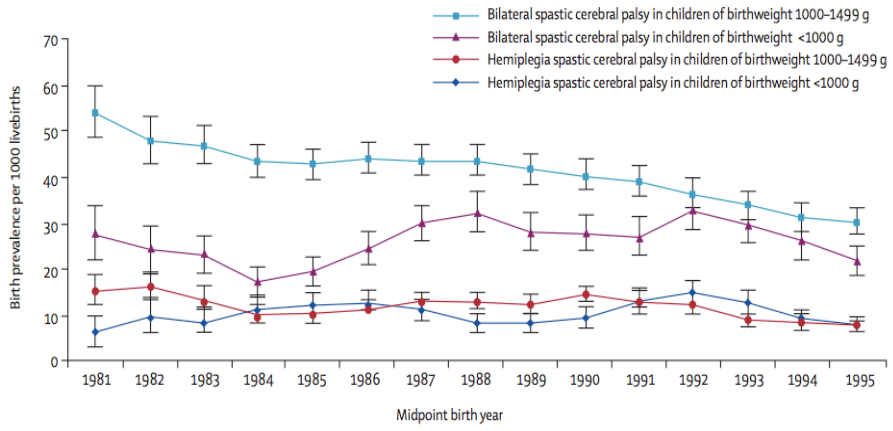


Fig.1.46. Birthweight-specific birth prevalence of bilateral and unilateral spastic cerebral palsy (3-year

moving average) from nine European centres, 1980–96 (Error bars=SE) (Platt et al. 2007)

1.4.1.1.1. Imaging correlates of cerebral palsy (CP)

In the European cerebral palsy study, a distinct pattern of brain injury was found on conventional MRI in preterm infants (born <34 weeks of gestation) with CP (Bax et al. 2006). White matter damage associated with immaturity, which included cystic PVL and periventricular haemorrhage, was the most common imaging correlate occurring in about 80% of these infants, followed by normal appearance on MRI, cortical and subcortical damage, focal cortical infarcts and basal ganglia damage (Bax et al. 2006). It should be noted that about 10% of the preterm children with CP had normal imaging findings. Children with spastic diplegia tended to have a different regional distribution of the white matter abnormalities from that of the children with spastic quadriplegia (Fig.1.47 and 1.48).

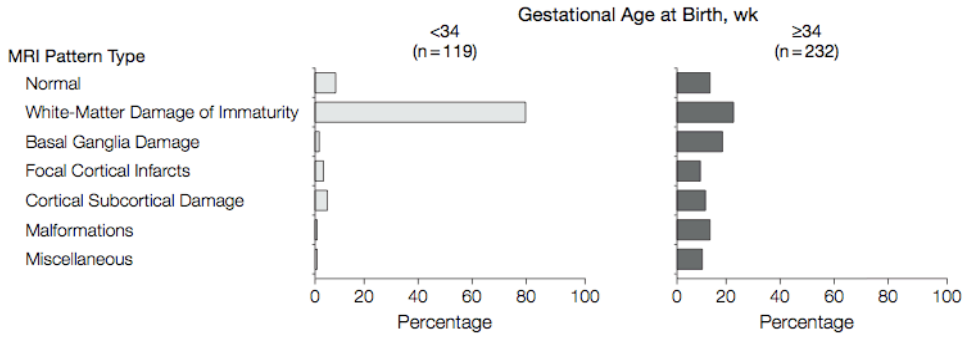


Fig.1.47. MRI pattern in infants with CP born < 34 weeks and >34 weeks of gestation (Bax et al. 2006)

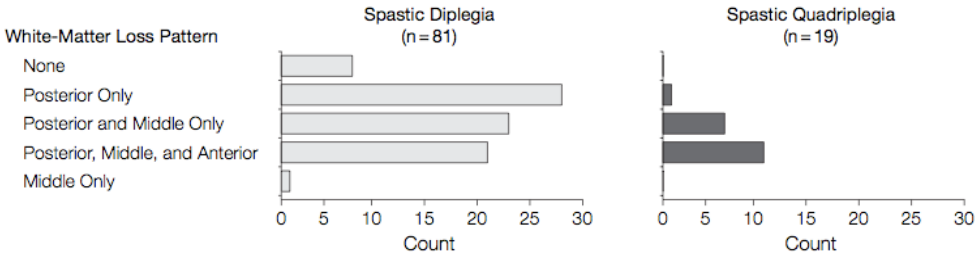
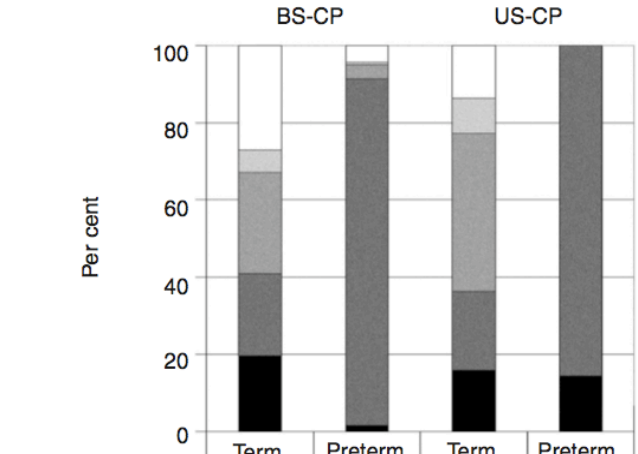


Fig.1.48. Location of white matter loss in spastic diplegia and spastic quadripelgia (Bax et al. 2006)

In a systematic review of studies using MRI in children with CP the same pattern of MR findings was described with predominant white matter injury in the preterm children with CP (Fig.1.49) (Krageloh-Mann and Horber 2007). 90% of children born preterm with CP had white matter injury in comparison to 20% of the term-born. White matter injury was either PVL or a consequence of intraventricular haemorrhage, or both. There seemed to be less children born preterm with CP and normal MR findings than in the study by Bax et al.



	Term 122	Preterm 186	Term 44	Preterm 14
Normal	33	8	6	0
Miscellaneous	7	1	4	0
Grey matter lesions	32	7	18	0
Periventricular lesions	26	167	9	12
Maldevelopments	24	3	7	2

Fig.1.49. MRI pattern distribution in two major CP subtypes. BS-CP, bilateral spastic cerebral palsy; US-CP, unilateral spastic cerebral palsy (Krageloh-Mann and Horber 2007)

The same MR brain injury pattern was very recently described in an Australian population. (Robinson et al. 2009)

In a conventional MRI study of preterm infants at term equivalent age, 10% of the preterm infants had CP: 67% of preterm infants with CP had severe, 21% moderate, 6% mild and 2% had no white matter abnormalities. More children with CP had grey matter abnormalities than normal grey matter (Woodward et al. 2006). Hence although conventional MR was predictive of CP, some of these infants had normal conventional imaging.

A DTI study of 28 preterm infants with CP with mean age of 5 years and ten months showed more severe injury in the posterior thalamic radiation pathways than in the

descending corticospinal pathways (Fig.1.50)

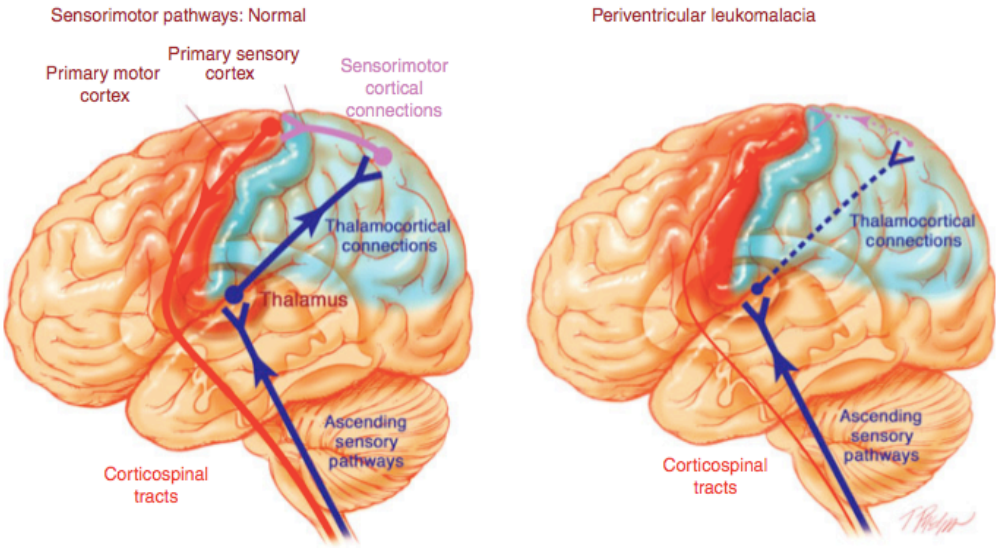


Fig. 1.50. Model of motor impairment associated with PVL. Image on the left shows normal sensorimotor pathways. The image on the right shows characteristic injury in the thalamo-cortical connections (blue dotted line) (Hoon et al. 2002)

1.4.1.2. Minor motor dysfunction

Motor skills of children born prematurely are affected even in the absence of CP (Jongmans et al. 1997; Foulder-Hughes and Cooke 2003; Hack et al. 2005; Davis et al. 2007). Indeed, motor difficulties or developmental coordination disorders are present in 30-40% of VLBW infants (Powls et al. 1995; Jongmans et al. 1997; Holsti et al. 2002; Davis et al. 2007). Fine motor deficits are described in 54% of preterm infants at 18 months, in 47% at 3 years and in 64% at 5 years (Goyen and Lui 2002). Developmental coordination disorder (DCD) refers to performance in daily activities requiring motor coordination that is markedly below the expected level, given the age and intellectual capacity. DCD prevalence in preterm infants has been reported as high as 10% (Davis et al. 2007), 30.7% (Foulder-Hughes and Cooke 2003) and 51% (Holsti et al. 2002). Serial motor assessment over time revealed a similar prevalence of DCD in apparently normal children: 42% in preterm infants compared to 8% for matched classroom controls (Goyen and Lui 2009). 30% of preterm infants had severe DCD compared with none in the control infants (Goyen and Lui 2009).

Motor performance and movement quality are impaired in VLBW children compared to a reference population and are related to the degree of neurological abnormalities (Schmidhauser et al. 2006). Particular impairments in timed motor performance were detected in the adaptive fine motor component with 44% of VLBW infants performing below normal (Schmidhauser et al. 2006). Preterm children with minor neuromotor

signs are at risk for neurocognitive impairment, in spite of average IQ (Korkman et al. 2008).

1.4.1.2.1. Imaging correlates of minor motor dysfunction

Motor performance and movement quality were assessed in 87 preterm infants at 6 years of age. Severity of PVL diagnosed using cranial ultrasound was associated with the adaptive fine motor component and intraventricular haemorrhage with the adaptive gross motor component (Schmidhauser et al. 2006). A population-based preterm cohort study showed a strong association between the size of the corpus callosum and motor function at school age: a smaller corpus callosum was associated with poorer scores on the Movement ABC (Rademaker et al. 2004). Brain abnormalities on conventional MRI detected at seven years of age were associated with more frequent minimal motor impairment (Abernethy et al. 2004). No conventional or quantitative MR studies correlating developmental coordination disorder with brain injury patterns in preterm infants could be found.

1.4.2. Cognitive Outcome

A meta-analysis of studies published between 1998 and 2008 on academic achievement, behavioural functioning and executive functions showed that preterm infants have moderate to severe deficits in academic achievement, attention problems, internalizing problems and poor executive functions, and during transition to adulthood these children tend to lag behind term-born peers (Aarnoudse-Moens et al. 2009). Cognitive deficits are very common in preterm infants (Msall et al. 1991; Saigal et al. 1991; Botting et al. 1998; Hack and Fanaroff 1999; Stewart et al. 1999; Wolke and Meyer 1999; Taylor et al. 2000; Vohr et al. 2000; Isaacs et al. 2001; Bhutta et al. 2002; Anderson and Doyle 2003; Ment et al. 2003; Marlow et al. 2005; Johnson et al. 2009). Whereas some infants have global cognitive impairments, others have specific deficits in perceptual-motor skills, learning and memory, and executive function (Whitfield et al. 1997; Taylor et al. 2000; Taylor et al. 2000). Most recently, the EPICure study showed that 11 years old children born below 26 weeks of gestation had significantly lower scores than their classmates for cognitive abilities, reading and mathematics (Johnson et al. 2009). A Bavarian study reported a relative risk of having serious cognitive impairment to no or mild impairment of 34.5 (95% CI, 8.5-139.5; $p<0.001$) for the preterm infants compared to the control infants. They tend to have cognitive deficits in more than one area; indeed, up to 38-44% have cognitive deficits in three or more areas such as general intelligence quotient (IQ), language comprehension and expression, calculation difficulties, articulation and pre-

reading skills (Wolke and Meyer 1999; van Baar et al. 2005). Preterm infants can have specific deficits in processing of simultaneous information to solve tasks, e.g. visual spatial recognition, pattern building and memory, and logical reasoning (Saigal et al. 1991; Wolke and Meyer 1999; Anderson and Doyle 2003; Marlow et al. 2005; Marlow et al. 2005; Mikkola et al. 2005; Wilson-Costello et al. 2005). In addition, VLBW infants are reported to make slower age-related gains on tests requiring perceptual-motor planning, attention shifting, and speed processing (Taylor et al. 2004). Recent reports suggest that these neuropsychological deficits persist into adolescence and adulthood (Botting et al. 1998; O'Brien et al. 2004; Allin et al. 2008; Johnson et al. 2009) and some studies even report a further decline in IQ until 15 years of age (Botting et al. 1998; O'Brien et al. 2004; Allin et al. 2008)

1.4.2.1. Intelligence Quotient (IQ)

Intellectual outcomes are impaired for VLBW infants; mean IQ is about 4-17 IQ points lower in VLBW infants than in control infants (Hall et al. 1995; Stjernqvist and Svenningsen 1999; Aylward 2002; Bhutta et al. 2002; Seitz et al. 2006). The deficit in IQ for ELBW infants is larger than that for VLBW infants (Hack et al. 1994; Hall et al. 1995). There seems to be a linear relationship between IQ and gestational age (below 32 weeks), with IQ decreasing with gestational age by an average of 2.5 points per week (Wolke D 2001; Bhutta et al. 2002) (Fig.1.51.). The mean IQ scores in the EPICure study correspond to the Bavarian study and confirm their prediction of a 2.5 point weekly fall in IQ. In the EPICure study EBLW boys had a group mean IQ score 10 points lower than the girls and were twice as likely to have impaired cognitive function (Marlow et al. 2005).

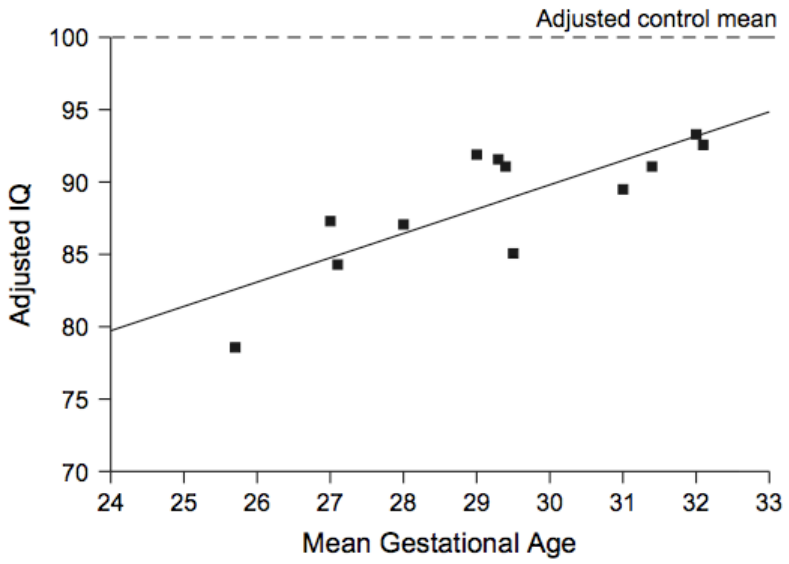


Fig. 1.51. Correlation between mean gestational age and mean IQ test score adjusted for comparison

with regressed control group mean (mean 100) for each cohort identified by Bhutta et al. (n=12, *r*=0.81, *p*=002) (Bhutta et al. 2002; Johnson et al. 2009)

1.4.2.1.1. Imaging correlates for Intelligence Quotient (IQ)

In 2002, Abernethy et al found no significant difference in IQ or attention deficits in preterm infants at 15-17 years of age with or without conventional MR-diagnosed brain injury (Abernethy et al. 2002). However, with quantitative volumetric analysis they found smaller volumes for the right caudate nucleus and left hippocampus in the preterm infants with low IQ (Abernethy et al. 2004). Skranes et al found no correlation between conventional MR abnormality and IQ in preterm children without disability at 6 years of age (Skranes et al. 1997) neither did the group of Krägeloh-Mann et al (Krageloh-Mann et al. 1999). Volumes of sensorimotor and midtemporal cortices were positively associated with full-scale, verbal and performance IQ in a cohort of 25 eight years old preterm children (Peterson et al. 2000). A voxel-based morphometry study showed the relationship between the decline in both verbal and performance IQ and brain regions over a period of 7 years of age and adolescence (Isaacs et al. 2004). Absolute IQ scores of 7.5-8 years old preterm children were related to areas in both the parietal and temporal lobes (Isaacs et al. 2004). Significant correlations between IQ scores and caudate volumes were seen, which persisted when children without visible lesions alone were considered (Abernethy et al. 2004). Higher absolute IQ scores were significantly associated with less grey matter, and more white matter in the parietal lobe (Isaacs et al. 2004). Positive correlations between cerebellar volume and full-scale IQ, performance and verbal IQ were seen in VLBW individuals by adulthood, but these did not persist after controlling for white matter volume (Parker et al. 2008). A recent MR study reported that total WM volume and corpus callosum area jointly explained 70% of IQ variance in adolescents born preterm at 16 years (Northam et al.).

1.4.2.2. Language

Language delay and deficits and articulation problems are common in preterm infants (Wolke 1999; Ment et al. 2003; Caravale et al. 2005; Mikkola et al. 2005; Luu et al. 2009). They can be associated with general cognitive impairment or they can be specific and independent of cognitive function or environmental disadvantages (Wolke 1999). Long-term adverse cognitive, language, and psychosocial outcomes have been reported in children with normal IQ but with specific language impairment; however, some studies of VLBW infants suggest that learning difficulties are not specific, but rather are more likely due to global deficits in cognitive function (Wolke

1998; Wolke et al. 2008). In a longitudinal neuropsychological study it was shown that VLBW infants had overall weaknesses on tests of motor and spatial constructional skills; even when estimated IQ was included as a covariate, the <750g group scored less well on tests of language processing and verbal working memory skills, verbal list learning, and perceptual-motor and spatial-organizational abilities (Taylor et al. 2004). A gradient of VLBW effect was noted; deficits relative to term-born controls were less pronounced for the 750-1499g group than for the <750g group (Taylor et al. 2004). In 6 year-old preterm infants scores for general intelligence, language comprehension, and expression, articulation and prereading skills were significantly lower compared with term peers (Wolke and Meyer 1999).

1.4.2.2.1. Imaging correlates of language outcome

A voxel based morphometry study of 15 years old preterm children showed that alterations in brain structure accounted for 28% of the variance of language scores (Nosarti et al. 2008). The results suggested that grey and white matter concentration, where between group anatomical differences rather than group membership differences (preterm vs control) were observed, were predictive of language and executive function scores (Nosarti et al. 2008). A more recent functional MRI study of 12 years old preterm children showed that children born prematurely and term controls engage the neural system for language differently (Schafer et al. 2009). Preterm children were found to have significant decreases in left frontal and bilateral temporal white matter volumes compared to term controls. Preterm children showed greater connectivity between traditional language areas and sensory motor areas but significantly fewer correlated areas within the frontal lobes when compared to term controls (Schafer et al. 2009).

1.4.2.3. Executive function

Executive functions describe psychological processes concerned with the control of thought and behaviour; linked closely together with these functions is working memory and attention. They refer to a collection of interrelated processes that are responsible for purposeful, goal-directed behaviour. Executive functions influence key academic and behavioural competencies and have therefore become a central area of investigation in cognitive development. In 1999, Luciana et al and Harvey et al reported working memory deficits in 7 to 9 years old preterm infants and they described differences in fine motor accuracy, spatial memory span, and pattern recognition between the preterm and control groups (Harvey et al. 1999; Luciana et al. 1999). In a recent outcome study, preterm born children revealed deficits across

all measures of executive function and memory, and severe brain injury diagnosed on cranial ultrasound was the most significant factor for these deficits (Luu et al. 2011).This is consistent with previous reports (Anderson and Doyle 2004; Marlow et al. 2007; Nosarti et al. 2007) and a meta-analysis showing higher rates of problems in executive function (Mulder et al. 2009). Executive impairments are found even when controlling for IQ or when excluding infants with CP (Klein et al. 1989; Hack et al. 1992; Taylor et al. 2000; Goyen and Lui 2002). Executive dysfunction is present in children with autism (Hughes 2002).

1.4.2.3.1. Imaging correlates with executive function

For executive function, the prefrontal cortex is considered important. However, executive dysfunction is not always associated with prefrontal pathology directly but may be related to network disconnections such as white matter injury or impairment to subcortical or posterior brain regions (Eslinger and Grattan 1993; Alexander and Stuss 2000). Brain imaging studies have confirmed and extended these findings by identifying a distributed network of areas in frontal and parietal cortex that appear involved in the allocation of attention, including the frontal eye field, anterior cingulate cortex, middle frontal gyrus, intraparietal sulcus and superior parietal lobule (Hopfinger et al. 2000; Kastner and Ungerleider 2000; Corbetta and Shulman 2002). A negative correlation was found in preterm adolescents at 15 years of age between FA in white matter and executive function (Skranes et al. 2009). The white matter areas that were involved corresponded to the inferior fronto-occipital fascicles and left cingulum, which both contain association fibers that are known to take part in executive function circuits (Skranes et al. 2009). The authors speculated that impairment in executive function in preterm infants might be influenced by disturbed connectivity between posterior brain regions and prefrontal cortices. The group of Woodward et al correlated the extent of white matter abnormalities seen at term equivalent age on conventional MR imaging with executive impairments at 4 years of age: only preterm infants who had mild to moderate white matter abnormalities had impairments in their executive functions at 4 years of age (Woodward et al. ; Edgin et al. 2008).

1.4.3. Behavioural outcome

1.4.3.1. Behavioural and emotional problems

Behaviour problems are common among preterm infants. Nearly every second VLBW adolescent has emotional or behavioural symptoms, as shown by Indredavik et al. Anxiety, relational problems and deficits in social skills are common (Indredavik

et al. 2004). The risk for psychiatric problems among VLBW adolescent persisted even after possible psychosocial confounders had been controlled for (Indredavik et al. 2004). Prevalences of psychiatric problems vary from 24% to 32% (Botting et al. 1997; Stevenson et al. 1999; Stjernqvist and Svenningsen 1999; Indredavik et al. 2004). Preterm infants at 3 and 5 years of age are reported to have a twofold higher prevalence for inattention/hyperactivity, emotional problems and peer problems than their peer control infants (Delobel-Ayoub et al. 2009). Poor cognitive performance was strongly associated with behavioural problems, but even when controlled for cognitive performance, the higher prevalence for behavioural problems was still significant (Delobel-Ayoub et al. 2009). In an Australian study, preterm infants showed significantly higher internalizing and dysregulation scores and lower competences scores than their peers did at 2 years of age (Spittle et al. 2009). Attention deficit hyperactivity disorder (ADHD) is diagnosed in16-23% of very low birth weight children, compared with 4-7% of normal controls (Lou 1996; Botting et al. 1997; Horwood et al. 1998; Wolke 1998; Bhutta et al. 2002; Indredavik et al. 2005). Extremely low birth weight children are particularly affected compared with the group of children born with a birth weight between1000 and 1500g (Bhutta et al. 2002). Parents usually rate behavioural problems of their children higher than the children or adolescents do themselves (Saigal et al. 2003). The prevalence of attention deficit hyperactivity disorder appears to decrease as children enter adolescence (Grunau et al. 2002; Saigal et al. 2003), suggesting a delayed development in these domains rather than a permanent cerebral dysfunction.

1.4.3.1.1. Imaging correlates of behavioural and emotional problems

Structural brain imaging of ADHD has been performed mainly in children. The main findings are: i. reduced intracranial volume (Durston et al. 2004) and reduced grey and white matter volume (Castellanos et al. 2002); ii. smaller prefrontal cortex volumes (Castellanos et al. 1996; Castellanos et al. 2001; Durston et al. 2004; Shaw et al. 2006); iii. abnormalities in the dorsal anterior cingulate cortex (Overmeyer et al. 2001); iv. abnormalities in the corpus callosum, particularly in the posterior regions (Hill et al. 2003); v. striate abnormalities (Lou 1996); and vi. abnormalities in the cerebellum, mainly the vermis (Castellanos et al. 2002; Hill et al. 2003). In a very large study of children and adolescents with ADHD, reductions of many brain regions were found, but after adjustment for cerebral volume was performed, only the difference in cerebellar volume remained significant (Castellanos et al. 2002). Smaller overall right cerebellar volumes were found in a group of 30 children with

ADHD (Durston et al. 2004). Reduced volumes in the temporal, parietal and occipital lobes have been reported in children with ADHD (Castellanos et al. 2002). Durston et al described a 9% reduction in the left occipital grey and white matter in children with ADHD (Durston et al. 2004). There seems to be a relationship of cerebellar and frontostriatal abnormalities in children with ADHD involving the cerebrocerebellar circuit with the ventral pontine nuclei, cerebellar cortex, deep cerebellar nuclei and thalamus via red nuclei (Seidman et al. 2005). These pediatric neuroimaging findings are important for the understanding of the structure-function relationship in preterm infants: cortical and cerebellar abnormalities are common in preterm infants and they might provide an explanation for the high incidence of attention deficits in preterm infants. Cerebellar growth in adolescence was assessed and related to behavioural outcome in preterm infants at 15 and then at 18 years of age (Parker et al. 2008). There was a significant negative correlation between change in cerebellar volume and several subscales of a General Health Questionnaire such as feeling worthless, feeling confident, feeling useful, decision-making capability, overcoming difficulties and concentration (Parker et al. 2008).

1.4.3.2. Autism spectrum disorder

Autism spectrum disorder (ASD) involves severe and pervasive impairment in thinking, feeling, language, and the ability to relate to others. Infants with ASD demonstrate deficits in social interaction, verbal and nonverbal communication and repetitive behaviours or interests. There are screening instruments to gather information about a child's social and communicative development; among them is the Modified Checklist for Autism in Toddlers (M-CHAT) (Robins et al. 2001). When the M-CHAT is used as a screen in unselected children during well-child care visits between age 16 and 30 months, 5.7% screen positive for ASD (Kleinman et al. 2008). Some follow-up studies of VLBW infants showed that VLBW infants are at higher risk for an autism diagnosis than their peer controls (Halsey et al. 1996; Elgen et al. 2002; Indredavik et al. 2005; Larsson et al. 2005; Schendel and Bhasin 2008). Schendel et al reported in a large population based study a two fold increased risk for autism in preterm birth <33 weeks of gestation and birth weight of < 2500g (Schendel and Bhasin 2008). The magnitude of risk from these factors varied according to gender and autism subgroup: girls had a fourfold increased risk for autism accompanied with mental retardation whereas no significantly increased risk was observed in boys for autism alone (Schendel and Bhasin 2008). Limeropoulos et al described a high prevalence of autism spectrum features among mean age of 21.9 months: 26% of preterm infants had a positive result on the autism-screening tool (M-

CHAT) (Limperopoulos et al. 2008). The positive M-CHAT results correlated strongly with the detection of internalizing behavioural problems and socialisation and communication deficits (Limperopoulos et al. 2008). Several factors seem to increase the risk for positive M-CHAT scores such as lower birth weight and gestational age, male gender, prenatal infection, greater illness, acuity based on SNAP-II scores, and abnormal MR studies (Limperopoulos et al. 2008). In a more recent study 21% of preterm infants born <28 weeks gestation screened positive on the M-CHAT; among the infants without motor, vision, hearing or cognitive impairment 10% screened positive (Kuban et al. 2009).

1.4.3.2.1. Imaging correlates of autism spectrum disorder

In a T2 relaxometry study, patients (9 years of age) with autism had T2 prolongation in cerebral white matter and had regional T2 differences in grey matter compared to controls. Furthermore, T2 was prolonged in associated white matter of the primary sensory association areas in the parietal lobes and in white matter of the visual association areas in the occipital lobes compared to controls (Hendry et al. 2006). Whole-brain cortical grey matter T2 was prolonged in children with autistic spectrum disorder (Petropoulos et al. 2006). In these studies there was no information about gestational age at birth or about conventional MR findings. Abnormal MRI findings at term-equivalent age were significantly associated with higher scores on the M-CHAT questionnaire. Interestingly, preterm infants with cerebellar haemorrhagic injuries were significantly more likely to have positive M-CHAT screen compared to those with isolated supratentorial injury (Limperopoulos et al. 2008). Cerebellar lesions have been described in autopsy or neuroimaging studies of children with autism (Baumann et al. 1988; Hashimoto et al. 1995; Courchesne et al. 2001; Limperopoulos et al. 2007).

1.5. Aims and hypothesis

The aims of this study were to assess white matter using quantitative MRI measures – T2 relaxometry and ADC - in a large cohort of preterm infants, and to determine the role of quantitative MRI in the assessment of white matter injury.

The hypotheses were as follows:

1.5.1. T2 relaxometry

1.5.1.1. T2 relaxation time is prolonged in preterm infants at term vs normal healthy infants

1.5.1.2. T2 relaxation time is prolonged in infants with DEHSI

1.5.1.3. T2 relaxation time is a more accurate predictor of outcome than ADC values

1.5.1.4. T2 relaxation time has a regional cephalo-caudal variation in values

1.5.1.5. T2 relaxation time correlates with neurodevelopmental outcome

1.5.2. ADC values

1.5.2.1. ADC is prolonged in preterm infants at term vs normal healthy infants

1.5.2.2. ADC is prolonged in infants with DEHSI

1.5.2.3. ADC is a less accurate predictor of outcome than T2 relaxomatry

1.5.2.4. ADC has a regional variation

1.5.2.5. ADC correlates with neurodevelopmental outcome

1.5.3. Quantitative MRI techniques (T2 relaxometry and ADC):

1.5.3.1. Are robust biomarkers of neurodevelopmental outcome at 2 years of age

1.5.3.2. Predict outcome more accurately than qualitative MRI eg DEHSI

Chapter Two

2. Methods

Ethical permission for this study was granted by the UCL/UCLH Research Ethics Committee (REC Ref No 03/0239) and informed parental consent was obtained.

2.1. Patients

Inclusion criteria: Preterm infants born at gestational age at birth of <32 weeks either at UCLH or within the perinatal network of UCLH between September 2005 and April 2008. Gestational age was calculated from the date of the last menstrual period and was confirmed with early ultrasound. Perinatal clinical data were recorded by me from the patient's and mother's notes (see appendix 1: minimal dataset). Healthy term control infants were recruited from the postnatal wards at UCLH.
Exclusion criteria: congenital malformation, ventilated or clinically unstable at time of corrected age of 38-42 weeks.

I did the recruitment of the patients: I approached the parents, explained the study and what it involves, answered their questions and consented them. I scheduled the scanning and organised it with the involved physicist and advanced neonatal nurse practitioner, or if the infant was discharged to another hospital or home I organised it with the involved clinicians or parents. Before we moved to the new UCLH building we had to transfer the preterm infants in the incubator to the radiology unit at UCLH.

2.2. MR imaging

MRI data were acquired on a Siemens (Erlangen, Germany) Avanto 1.5T scanner using the Siemens CP extremity coil. On the day of scanning, I was responsible for the safety check of the infants, prescribed the sedation and planned the feeding times in accordance with scanning times. Preterm infants were sedated with an oral dose of 50mg/kg chloral hydrate (500mg/5ml); the control infants were scanned during natural sleep and were not sedated for scanning. I was responsible for transferring the infants by ambulance to the main UCLH building. At the radiology unit we administered the sedation, wrapped the infants within a blanket, put on ear protection including ear plugs (attenuation: 24 dB; Earsoft; Aearo, Indianapolis, IN) and Minimuffs (attenuation: 7 dB; Natus, San Carlos, CA), then pulse oxyimetry and ECG monitoring were put on the infants and we placed them once they had fallen asleep in a transparent MR compatible pod. A neonatal nurse who was trained by me in MRI procedures was present throughout the scanning. Observations during the scanning were recorded on an observational sheet devised by me. A physicist was

responsible for running the scanner. I checked the quality of the acquired images and decided whether sequences needed to be repeated. I was responsible for transferring the infants back to the neonatal unit and for informing the parents about how the scanning went. The imaging findings were discussed within the weekly radiology meeting and after that, I informed the parents about the findings and explained them.

2.2.1. Conventional imaging

T1 weighted 3D-FLASH images (TE/TR=6.06/17ms; 160 1mm slices; field of view (FOV) 200mm x 200mm; data matrix 256 x 200, flip angle 21˚ and bandwidth 100 Hz/pixel) and T2-weighted fast spin echo images (TR=5.91s, 23 3mm axial slices thickness, FOV 210mm x 157.5mm, data matrix 512 x 192, 11 echoes and an effective echo time TEeff=110ms; bandwidth 65 Hz/pixel) were acquired before T2 relaxometry and diffusion sequences.

2.2.2. T2 relaxometry

T2 relaxometry: nineteen preterm infants had T2 relaxometry using a spin-echo (SE) sequence (TR/TE = 1.55s / 10, 100 and 300ms; FOV 160mm x 120mm; data matrix 192 x 144; bandwidth 80Hz/pixel; five 5mm axial slices positioned, one of which was at the level of the CSO). The remaining preterm infants had T2 relaxometry using the manufacturer's EPI SE sequence (TE = 89 and 200ms; 23 axial slices; FOV 210mm x 157.5mm, data matrix 128x96) (Fig.1a-c). In 13 infants both T2 relaxometry sequences were obtained. After inspection for motion artefacts, maps of T2 were calculated offline with software developed in Matlab 6.0 (Mathworks, USA) by fitting a decreasing exponential function to the signal intensity as a function of echo time.

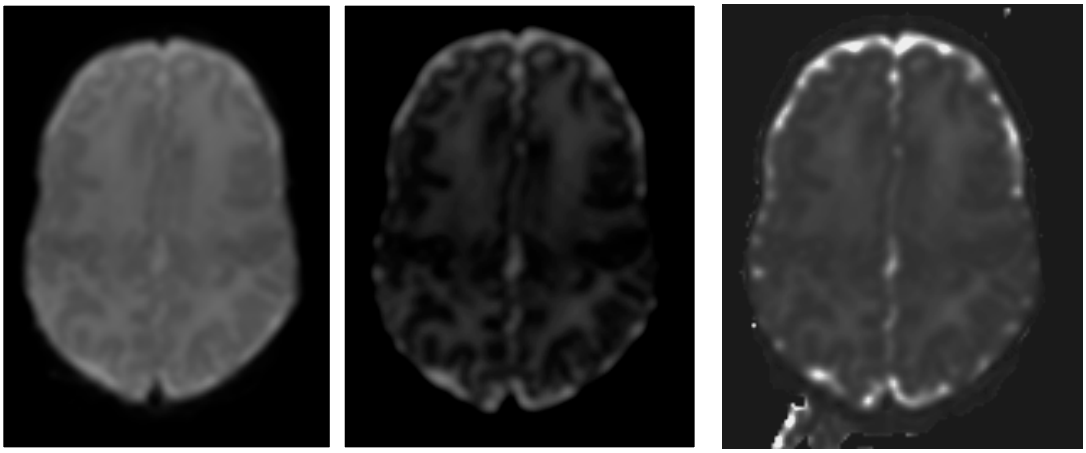


Fig. 2.1a-c. EPI T2 images with (a) TE 89 ms, (b) TE 200 ms and (c) the corresponding T2 map. Slices shown are at the level of the centrum semiovale. As TE increases there is more contrast between white and grey matter.

2.2.3. Diffusion weighted and tensor imaging

Diffusion weighted imaging (DWI) used the Siemens double spin echo EPI sequence (TR 4.2 s; TE 118 ms, twenty three 3mm thick axial slices; FOV 230 mm x 172.5 mm; data matrix 128 x 96; bandwidth 1260Hz/pixel, 5 averages) for all preterm and control infants. Three orthogonal diffusion encoding directions were used with b 50 and 600 s/mm^2; ADC maps were calculated using a 2-point log-linear fit. ADC, FA, RA and eigenvalue maps were calculated offline using software DTI studio Version 2.4.01 (Johns Hopkins University, Baltimore, USA). Images with motion artefacts were excluded from the analyses.

2.3. Neurodevelopmental Follow-up

2.3.1. Neurological examination

Level	**GMFCS**
1	Children can walk without restrictions but have limitations in more advanced gross motor skills (eg, coordination and balance)
2	Children can walk without assistive devices but have limitations in walking outdoors and in the community (eg, running and jumping)
3	Children can walk with assistive mobility devices and have limitations in walking outdoors and in the community
4	Children with limitations in self-mobility; these children use power mobility outdoors and in the community.
5	Children have severely limited self-mobility, even with the use of assistive technology

Table.2.1. Gross Motor Function Classification System

Abnormalities in tone, posture and movement consistent with CP were recorded. Severity of CP was defined with the gross motor function classification (GMFCS) published by Palisano (Palisano et al. 1997). The neurological examination was done either by Dr Angela Huertas Ceballos or me.

2.3.2. Neurodevelopmental assessment

I identified the infants that were due for neurodevelopmental assessment at one and two years of corrected age and checked with the clinical FU coordinator Lynn Collier whether an appointment was given or needed to be given. Preterm infants born below 32 weeks are seen clinically at one and two years of corrected age at UCLH as part of the clinical follow-up program led by consultant Dr. Angela Huertas-Ceballos. There was no research dedicated FU programme at the time of my PhD. I wrote letters to invite the parents for FU assessment and would call them by phone to

ensure they would come. If an appointment was not attended by the parents, I tried to reach them by phone and Lynn Collier would send them an invitation letter for another appointment. I offered home visits to the parents and organised them. Sam Johnson would accompany me to perform the neurodevelopmental assessment at home while I performed the neurological examination.

The infants were assessed using the Bayley Scales of Infant Development (BSID III-2005) which is a standardised scale used to identify deficits in very young children (1-42 months) across five major developmental domains: cognition, language, motor skills, adaptive behaviour, and social-emotional. The scales give standardised index scores and sub-test scaled scores for each domain. The neurodevelopmental assessments were performed by Dr Angela Huertas-Ceballos, Betty Hutchinson (Honorary Consultant Developmental Therapist) and Sam Johnson (Non-clinical Lecturer in Academic Neonatology, Psychology). The assessors were blinded to the MR findings. For this study, composite cognitive, language and motor scores, and fine and gross motor scaled scores as well as receptive and expressive scaled scores were used for analyses.

2.4. Data analysis

2.4.1. Patients

I entered the data recorded on the perinatal data of the minimal datasets in a SPSS database and analysed the data using parametric or nonparametric tests depending on data normality. Demographic maternal and neonatal characteristics were summarised in tables. Infants were grouped into sub-groups depending on their clinical characteristics such as for example chronic lung disease defined as oxygen requirement at corrected 36 weeks of age, inotropic use, presence of chorioamnionitis etc.

2.4.2. Conventional images

Axial T2 weighted images, axial and sagittal T1 weighted images were assessed by Dr Rox Gunny, Consultant in Paediatric Neuroradiology at Great Ormond Street Hospital and by me, according to the MR proforma developed by me for this study (appendix 2: MRI proforma). The presence of punctate white matter lesions (PWML) and cystic WM lesions, germinal matrix or intraventricular haemorrhage, intraparenchymal haemorrhagic lesion (HPI), cerebellar haemorrhages, deep grey matter abnormalities and signal intensity abnormalities of the hemispheric and cerebellar WM was noted. Myelination in the corticospinal tract and maturation of the

cortical folding were assessed. Volume of WM was visually assessed by the size of the ventricles and size of corpus callosum. The findings were coded and entered into the SPSS database. The infants were grouped depending on their MR findings.

2.4.3. Quantitative MR measurements

2.4.3.1. Sequence stability and accuracy

DWI and EPI T2 relaxometry accuracy and stability were assessed using distilled water and a 10mg $MnCl_2$/L water phantom. These studies were performed by Enrico deVita and me.

2.4.3.2. T2 relaxometry

I assessed images for movement artefact; those degraded by motion artefact were excluded. On the least T2 weighted image available (resolution interpolated to 0.45 mm x 0.45 mm) circular regions of interest were positioned by me in 20 regions per hemisphere and then transferred to the T2 maps using ImageJ (Fig.2.2a-c and 2.3a-c): mean T2 was then calculated for each ROI. T2 values were transferred into an excel database and then into the SPSS database. Paired t tests were done to evaluate whether there were statistical significant differences in T2 between hemispheric measurements. If there was no statistical difference (p value < 0.05) the mean of both hemispheric measurements was calculated and used for further analysis. If there was a significant difference between both hemispheres T2 of of each hemisphere was used for further analysis. Linear regression was performed to evaluate whether there was a relationship between T2 measurements and any other quantitative MR measurements such as ADC, FA and eigenvalues, age at birth or scanning, any clinical data, MR variables and outcome measures. After grouping infants depending on their outcome, stepwise binary logistic regressions were performed to evaluate which T2 measurements were independent risk factors for outcome. To test interobserver agreement of ROI placement Enrico deVita and I drew ROIs in 20 infants. ROI placement was tested by calculating the kappa statistics, defining the following ranges: 0.00 “poor agreement”; 0.00 to 0.20 “slight”; 0.21 to 0.40 “fair”; 0.41 to 0.6 “moderate”; 0.61 to 0.80 “substantial” and 0.81 to 1.00 “almost perfect agreement” (Landis and Koch 1977).

Bland-Altman analysis was performed to test the reliability of the two different T2 techniques SE and EPI T2 relaxometry.

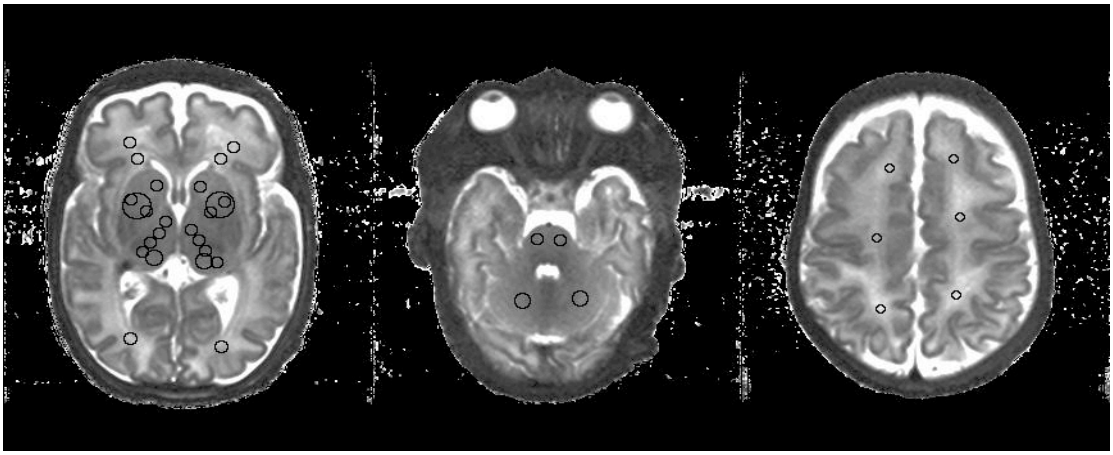


Fig.2.2a-c. SE T2 maps with placements of ROIs at the level of the cerebellum, the level of the basal ganglia and the level of the centrum semiovale

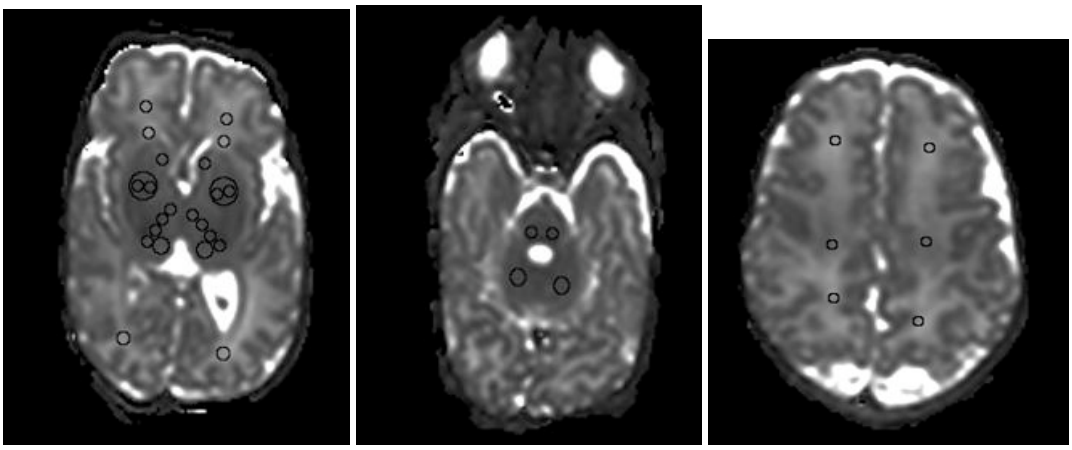


Fig. 2.3a-c. EPI T2 maps with placements of ROIs at the level of the cerebellum, the level of the basal ganglia and the level of the centrum semiovale

2.4.3.3. Diffusion weighted and tensor images

Images degraded by motion artefacts were excluded. T2 ROIs were positioned onto ADC maps using ImageJ and ADC values were calculated, copied into an excel sheet and transferred to the SPSS database. In the instances when the infant had moved between T2 relaxometry and diffusion acquisition, the ROIs were re-drawn on the b0 images and transferred to the ADC maps. FA and eigenvalues maps were created by me using DTI studio software. ROIs for the posterior limb of the internal capsule (PLIC), genu and splenium of the corpus callosum and the WM of the CSO were drawn by me on FA maps and then transferred onto the eigenvalue maps. Calculated values were entered into the SPSS database. Linear regression was performed to evaluate whether there was a relationship between ADC, FA or eigenvalue measurements and any other quantitative MR measurements such as T2 measurements, age at birth or scanning, any clinical data, MR variables and outcome

measures. After grouping infants depending on their outcome, stepwise binary logistic regressions were performed to evaluate which ADC, FA and eigenvalue measurements were independent risk factors for outcome

2.4.4. Neurodevelopmental outcome

Composite scores for cognition, language and motor tests and scaled scores for receptive communication, expressive communication, fine and gross motor were used for outcomes in the analyses. Furthermore, preterm infants were grouped into those with cognitive, motor and language scores above and below 85 for logistic regression analysis.

2.5. Statistical analyses

All data was analysed with SPSS statistical software. Results were significant at a level of $p \leq 0.05$. Exploratory analyses of outcomes were undertaken. Parametric or nonparametric tests were used depending on the nature of the data. Pearson correlation was done to evaluate the correlation between clinical data and outcome measures. These were reported with pearson correlation coeffcients (r) and p values. Stepwise multiple linear regressions between clinical variables and outcome measures were then performed. Linear regressions were used to test the correlation between MR outcome and composite cognitive, motor and language scores, fine and gross motor scaled scores, and receptive and expressive language scaled scores. First univariate analyses were performed, and then significant variables were included into a multiple linear regression. Regression coefficients with 95%CI and p values were used to show the results. After grouping the infants into those with composite scores above or below 85, logistic regression analyses were performed in order to evaluate independent risk factors for outcome. Binary logistic regression was used with a stepwise method, Exp (B) and 95% CI are shown to illustrate the results. I did all statistical analyses.

Chapter Three

3. Results

3.1. Preterm and term infants

3.1.1. Demographical and clinical data of preterm infants

80 preterm infants were recruited in 17 months (September 2006 to February 2008) from the neonatal unit at UCLH. Table 3.1 illustrates the maternal characteristics: almost half of the mothers were primigravida with a mean age (SD) of 32.36 (5.6) years. Pregnancy was complicated by hypertension in 13.5% and by preeclampsia in 11.3%. Onset of labour was spontaneous in 70% and induced in 2.5%. In 27.5% no labour activity was present on admission. 50% were vaginal deliveries and 50% caesarean section. 90% of the mothers received antenatal betamethasone for lung maturation. Rupture of membranes occurred in 50% of the mothers: in the majority of the cases (37.5%) this happened within 72 hours of birth; however in 12.5% it occurred more than 72 hours prior to delivery. CRP was normal in only 8.8% of the mothers within 72 hours of birth. Only 15% of all available placental histology (n=66) was normal. Chorioamnionitis with or without funisitis was the most common histological finding occurring in 30% and 11% respectively.

Maternal and pregnancy related details	
Maternal age in years (mean, SD)	32.36 (5.68)
Primigravida	47.5%
Assisted conception	10%
No smoking during pregnancy	85%
Hypertension during pregnancy	13.8%
Prae-eclampsia • Yes • With seizures • No	 11.3% 1.3% 87.5%
Use of antibiotics within 2 weeks prior to delivery in	35%
Use of antitocolytics within two weeks prior to delivery in	17.5%
Antenatal betamethasone • Given • Not given • Don't know	 91% 4.5% 4.5%
Maternal temperature >38° • <24 hours pre and post delivery • >2 weeks prior to delivery	 15% 1.3%
Highest maternal CRP within 2 weeks prior to delivery	44.5 (1.00-314.60)

(median, range)	
Highest maternal white cell count within 2 weeks prior to delivery (median, range)	15.34 (6.75-65.80)
Placenta praevia: yes	1.3%
Liquor volume • Normal • Increased • Decreased • Not known	 80% 5% 14% 1%
Rupture of membrane • <24 hours • 25-48 hours • 49-72 hours • >72 hours • No rupture	 22.5% 10% 5% 12.5% 50%
Onset of labour • Spontaneous • Induced • No labour	 70% 2.5% 27.5%
Mode of delivery • Vaginal, no instruments • Caesarean section in labour • Caesarean section no labour	 50% 20% 30%
Placental histology (n=66) • Normal • Chorioamnionitis • Chorioamnionitis and funisitis • Infarcts • Excess of fibrin and syncitial knots • Infarct and excess of fibrin and syncitial knots • Other	 12 (18%) 7 (11%) 20 (30%) 5 (7%) 8 (12%) 3 (5%) 11(17%)

Table 3.1. Maternal and pregnancy related details

The infants' demographic, perinatal and neonatal data are summarised in Table 3.2-3.4.

One of the main characteristics of this cohort is the wide range of gestational age at birth including the very extreme preterm infants born at 23 weeks of gestation. 61% (n=49) of the included preterm infants were born below 28 0/7 weeks and of those 26% (n=14) below 24 0/7 weeks of gestation. 49% off all infants' birth weight was between the 10th and 90th centile, 14 infants' birth weight was below the 2nd birth

weight centile (Table 2). There were almost equal numbers of female and male infants with 54% female and 46% male infants. No correlation between gender and birth weight or head circumference or gestation at birth or race could be found.

Demographic characteristics	**N=80**
Place of delivery • UCLH • Ex-utero transfer	 89% 11%
GA in weeks (mean (SD), range)	27.41 (2.81), 23.4- 32.3
BW in grams (mean (SD), range)	1008.63 (388.60), 454- 2470
BW centile • <0.4th • >0.4th to 2nd • 2nd to 9th • 10th to 90th • >90th	 6 8 14 49 3
HC at birth in cm (n=71) (mean (SD))	32.0 (5.6)
Gender (female)	43 (54%)
Singleton	63.7%
Race • Caucasian • Black • Asian • Mixed	 38% 18% 15% 12%

Table 3.2. Demographics of all preterm infants

Most infants required intubation, surfactant administration and ventilation at birth. Median APGAR at 1 and 5 minutes were 5 and 9 respectively (Table 3). Highest median lactate within the first 72 hours after birth was 3.14 ranging from 0.96 to 12.5 mmol/l. CRP and platelets count within the first 72 hours were noted (Table 3.3). Median CRP was 6.6mg/l ranging between 1.00 and 212.0mg/l and median platelet count 115/µl (Table 3.3). No correlation between CRP and platelet count could be found. Three infants had positive blood cultures within 72 hours after birth.

Characteristics at delivery	**N=80**
Presentation at delivery • Cephalic • Breech • Other • Don't know	 46.2% 18.8% 12.5% 22.5%
Meconium stained liquor in	2.5%
Resuscitation at delivery	

• Minor (suction, O_2) • Major (intubation, surfactant) • None	11.25% 76.25% 12.5%
Apgar at one minute (median (range))	5.5 (1-10)
Apgar at 5 minutes (median (range))	9 (1-10)
Lowest pH within first 24 hours (mean (SD))	7.23 (0.11)
Highest BE within first 24 hours (mean (SD))	-6.57 (4.02)
Highest lactate (mmol/l) within first 24 hours (median (range))	3.14 (0.96-12.5)
CRP (mg/l) within first 72 hours (median (range))	6.6 (0.1-212.20)
Lowest Platelets (/µl) within first 72 hours (median (range))	115.00 (11.9-300)

Table 3.3. Characteristics of the delivery and resuscitation

Neonatal details are shown in Table 3.4 .17 infants were never ventilated, 45 were ventilated predominately (>80% of the ventilated time) with conventional ventilators and 18 infants were ventilated predominantly with HFOV. Pneumothorax complicated the respiratory course in 10 infants of which two were never ventilated and in 4 each with predominantly conventional ventilation or HFOV (table 3.4). Hence, being ventilated correlated with the occurrence of pneumothorax (*p=0.03*). Median ventilation days were 6.6 days ranging from never ventilated to 108 days of ventilation. 49% of the infants were in oxygen at term equivalent age. Median time to room air was 27.5 days ranging from always having been in room air to 159 days of additional oxygen requirement. 11 infants were discharged home in oxygen.

Neonatal characteristics	**N=80**
Acute respiratory syndrome • Present • Not present	 86.25% 13.75%
Pneumothorax • Present • Not present	 12.5% 87.5%
Inotropic use • Yes	 36.3%
Patent ductus arteriosus • Yes, but no treatment • Surgery only • Medication only • Both • No PDA	 15% 15% 19% 9% 42%
Indomethacin given	25%
Iboprufen given	7.5%

Necrotizing enterocolitis • Proven clinically and radiologically • Suspicious • None	 17.5% 18.8% 63.7%
Oxygen at 28 days of age	55 (55%)
Oxygen at 36 weeks of age	47 (59%)
Oxygen at term equivalent age	39 (49%)
Ventilation days (median, range)	6 (0-108)
Retinopathy of prematurity • Grade I-II • Grade III and worse • None	 28.8% 13.8% 51.2%
Abnormal movements noted at any time • Yes (n=5)	 6.3%
Seizures confirmed with EEG • Yes (n=2) • Anticonvulsants (n=2)	 2.5% 2.5%

Table 3.4. Neonatal characteristics of the cohort

Table 3.5 compares the characteristics of preterm infants born below 28 weeks to preterm infants born >28 weeks of gestation. As expected, preterm infants born below 28 weeks showed a higher incidence of oxygen requirement at 28 days, 36 weeks and at term, required more frequently inotropes for blood pressure support, and had PDA, NEC and ROP more frequently (Table 3.5). Chorioamnionitis with funisitis was more commonly found in placental histology of preterm infants born below 28 weeks compared to those born above 28 weeks (Table 3.5).

Demographics	**Preterm infants ≤28 weeks (n=49)**	**Preterm infants ≥28 weeks (n=31)**
BW in grams (mean (SD)	813 (196)	1317 (418)
Gender (female)	30 (61%)	13 (42%)
Singleton	34 (70%)	17 (55%)
Race • Caucasian • Black	 23 (47%) 6 (12%)	 15 (48%) 12 (39%)
Oxygen at 28 days of age	44 (89%)	11 (35%)
Oxygen at 36 weeks of age	38 (76%)	9 (29%)
Oxygen at term equivalent age	33 (67%)	6 (19%)
Inotropic use	24 (49%)	6 (14%)
PDA	35 (71%)	10 (32%)
NEC	14 (29%)	0
Chorioamnionitis with funisitis	16 (33%)	4 (13%)

ROP any grade	31	3

Table 3.5. Characteristics according to gestational age above or below 28 weeks of gestation

3.1.1.1. Demographical and clinical data of term control infants

Nine term control infants were recruited with mean gestational age at birth of 38.3 (1.29 weeks and mean birth weight of 2952 (412) grams. Their mean age at MR scanning was 40.1 (1.6) weeks. They were all born by vaginal delivery with normal adaption. None of them was admitted to the neonatal unit and they all had normal neurological examination.

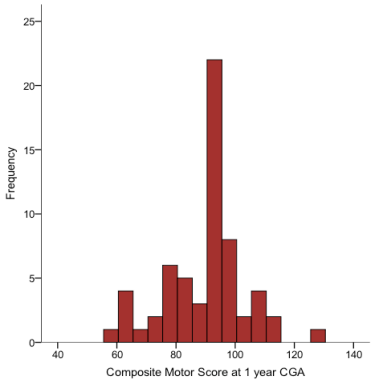
3.1.2. Outcome at one year

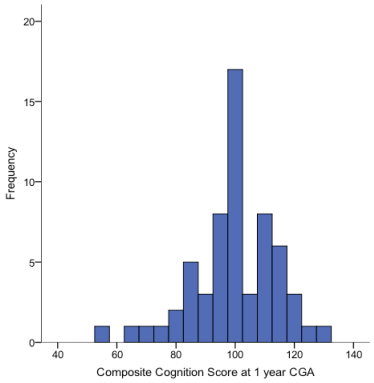
61/80 (76.3%) had follow-up (FU) assessment at one year corrected gestational age. Mean age at FU assessment was 12 months and 10 days (range 9 to 15 months). Six families moved abroad and 11 families did not attend any appointment despite several phone calls and written invitations. Table 3.6 shows the differences between the infants who had FU at one year CGA and those who did not have 1-year assessment. As CLD, inotropic use and BW are significantly related to gestational age at birth, it is not unexpected that the younger infants who had better FU attendance were smaller, had more often PDA, CLD and GMH-IVH.

	FU (n=61)	**No FU (n=19)**
Gestational age at birth (in weeks)	26.7 (2.5)	29.6 (2.5)
Birth weight (in grams)	943 (349)	1220 (439)
Gender (female)	48%	73%
Inotropic use	43%	21%
CLD	70%	26%
PDA	59%	47%
NEC	23%	none
GMH-IVH	23%	5%
Cystic WM lesions	25%0	17%
DEHSI	80%	68%
PWML	34%	26%

Table 3.6. Neonatal and imaging characteristic differences between infants who did attend and those who did not attend FU

The following histograms show the normal distribution of composite cognitive, language and motor scores, receptive and expressive communication scaled scores and gross and fine motor scaled scores (Fig. 2.1a-f).





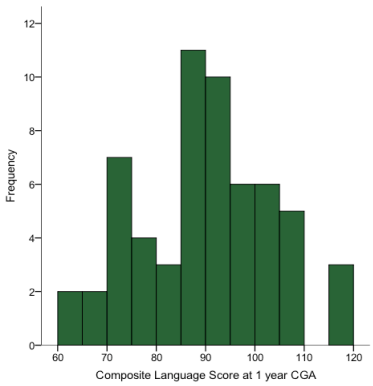
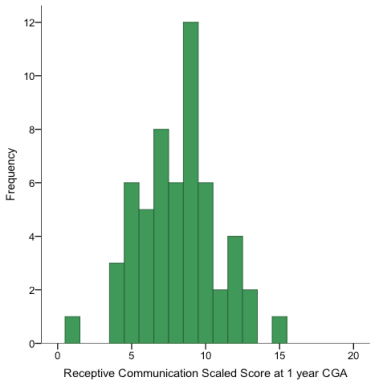


Fig.3.1a-c. Histograms showing normal distribution of composite motor (a), cognition (b) and in (c) language scores at corrected gestational age of one year.



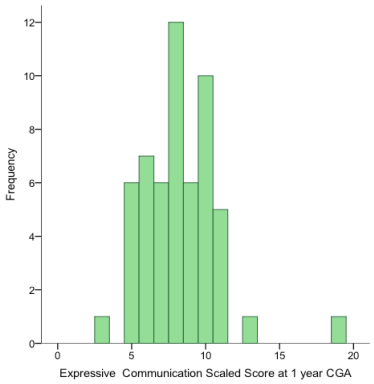
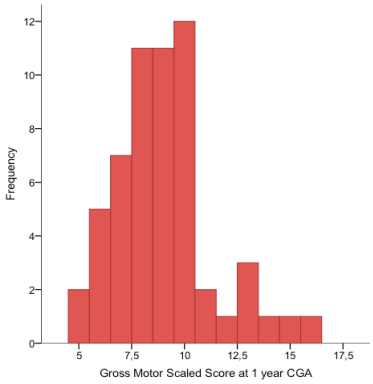


Fig.3.1c and d Histograms showing the distribution of receptive and expressive scaled scores at corrected gestational age of one year



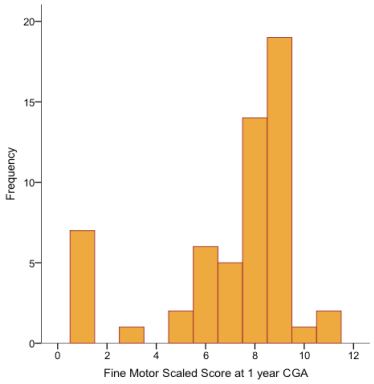


Fig.3.1e and f. Histograms showing the distribution of gross and fine motor scaled scores at corrected gestational age of one year

Table 3.7 shows the mean (SD) cognitive, motor and language scores of all preterm infants at one year of corrected gestational age.

	All preterm infants (n=61)
Composite Cognitive Score	99.7 (14.2)
Composite Motor Score	89.7 (13.5)
Composite Language Score	90.0 (12.9)
Receptive Communication Scaled Score	8.1 (2.7)
Expressive Communication Scaled Score	8.3 (2.5)
Gross Motor Scaled Score	9.0 (2.3)
Fine Motor Scaled Score	7.1 (2.7)

Table 3.7. Mean (SD) cognitive, motor and language scores

Composite cognitive scores correlated with composite motor scores (Fig.3.2) (*p<0.01*) and there was a positive correlation between composite language and composite cognitive scores (*p<0.01*).

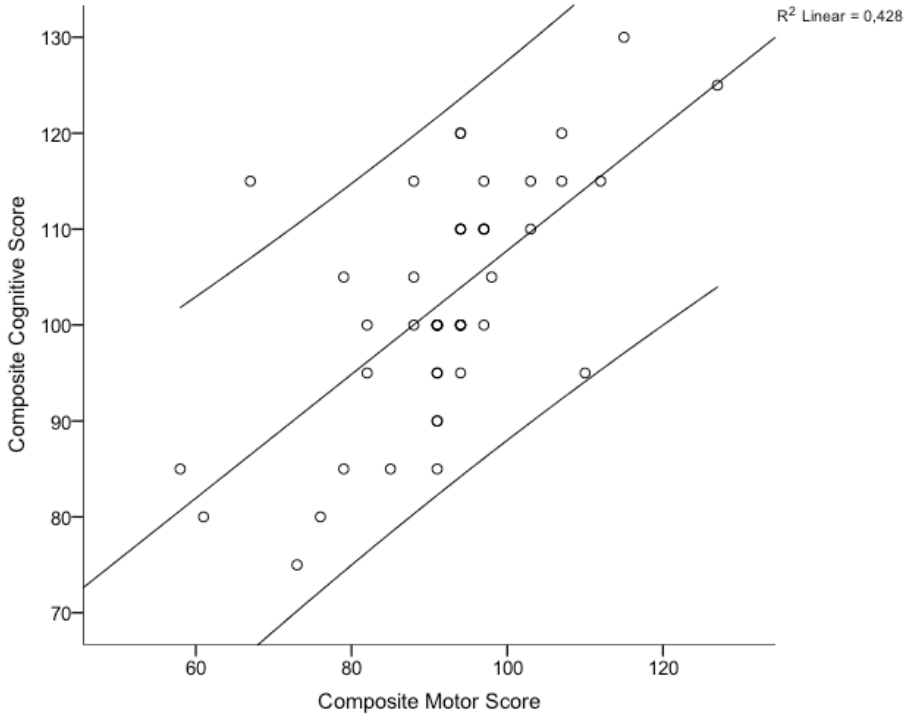


Fig.3.2. Scatterplot showing linear correlation between composite cognitive and motor scores at one year corrected gestational age. Inner line represents the linear fit, outer lines CI 95%

Cognitive scaled scores were correlating to some degree with receptive communication scaled scores and with expressive communication scaled scores. There was poor correlation between gross and fine motor scaled scores and better correlation between receptive and expressive communication scaled scores.

3.1.2.1. Correlation of one year outcome with clinical data (Pearson correlations (r) and multiple linear regression)

Composite cognitive score were associated with gestational age at birth ($p<0.01$, $r= 0.95$), birth weight ($p<0.01$, $r= 0.56$), race ($p<0.01$, $r= 0.3$), postnatal hydrocortisone administration ($p=0.03$, $r= 0.43$), inotropes ($p<0.01$, $r= 0.46$), oxygen requirement at 28 days ($p=0.01$, $r= -0.38$) and at 36 weeks corrected gestational age ($p=0.01$, $r= -0.36$) and chorioamnionitis ($p=0.01$, $r= 0.35$). No correlation was found with gender, PDA, NEC and chorioamnionitis with funisitis. On multiple linear regression only birth weight ($p=0.01$, regression coefficient (95%CI) 0.018. (0.03 to 0.1)) and chorioamnionitis ($p=0.03$, regression coefficient (95%CI) –11.5 (-21.9 to -0-12)) remained significantly correlated with composite cognitive.

Composite motor scores were associated with birth weight ($p<0.01$, $r= 0.38$), gestational age at birth ($p<0.01$, $r= 0.96$), inotropes ($p<0.01$, $r= 0.28$), oxygen requirement at 28 days ($p<0.01$, $r= 0.29$) and at 36 weeks corrected gestational age ($p<0.01$, $r= 0.43$) and chorioamnionitis ($p=0.02$, $r= 0.29$). No correlation was found with gender, race, postnatal hydrocortisone administration, PDA or NEC. On multiple linear regression chorioamnionitis remained significantly correlated with composite motor score ($p=0.03$, regression coefficient (95%CI) -11.8 (-22.6 to -0.9)). Gross motor scaled score correlated with GA ($p<0.01$, $r= 0.36$), BW ($p<0.01$, $r= 0.32$), oxygen requirement at 28 days ($p=0.01$, $r= -0.43$), race ($p=0.02$, $r= -0.3$) and at 36 weeks corrected gestational age ($p=0.02$, $r= -0.36$) and chorioamnionitis ($p<0.01$, $r= 0.45$). No correlation was found with PDA, NEC, inotropes, chorioamnionitis with funisitis and postnatal hydrocortisone administration. On multiple linear regression race and chorioamnionitis remained significantly related to gross motor scaled score ($p<0.01$, regression coefficient (95%CI) -4.7 (-5.6 to -2.4)).
On univariate and multiple linear regression inotropes was the single significant correlate with fine motor scaled score at one year CGA.

Composite language scores were associated with birth weight ($p=0.01$, $r= 0.33$), inotropes ($p=0.03$, $r= -0.28$), postnatal hydrocortisone administration ($p<0.01$, $r= -0.37$), oxygen requirement at 28 days ($p<0.01$, $r= -0.39$) and at 36 weeks corrected gestational age ($p=0.01$, $r= -0.43$) and chorioamnionitis ($p<0.01$, $r= 0.34$). No correlation was found with GA, race, PDA, NEC or chorioamnionitis with funisitis. On multiple linear regression chorioamnionitis ($p=0.35$, regression coefficient (95%CI) -- 9.9 (-19.0 to -0.7)) and postnatal hydrocortisone administration ($p=0.04$, regression

coefficient (95%CI) 9.0 (0.5 to 17.5))) remained significant related with composite language score at one year CGA.

Receptive communication scaled score was associated with birth weight (p=0.03, r= 0.03), NEC (p<0.01, r= -0.37), postnatal hydrocortisone administration (p<0.01, r= -0.37), chorioamnionitis (p=0.01, r= 0.34), and oxygen requirement at 28 days (p=0.02, r= -0.3) and at 36 weeks corrected gestational age (p=0.03, r= -0.28). No correlation was found with GA, race, PDA, inotropes and chorioamnionitis with funisitis. On multiple linear regression chorioamnionitis (p=0.05, regression coefficient (95%CI) -1.9 (-3.9 to 0.003)), NEC (p=0.01, regression coefficient (95%CI) 1.96 (0.4 to 3.4)) and postnatal hydrocortisone administration remained significantly correlated with receptive communication scaled score.

Expressive communication scaled score was associated with birth weight (p=0.04, r= 0.28), postnatal hydrocortisone administration (p=0.02, r= -0.31)., chorioamnionitis (p<0.01, r= 0.43), and oxygen requirement at 28 days (p<0.01, r= -0.42). and at 36 weeks corrected gestational age (p<0.01, r= -0.38). No correlation was found with GA, PDA, NEC, chorioamnionitis with funisitis or with inotropes. On multiple linear regression analysis chorioamnionitis (p=0.02, regression coefficient (95%CI) -11.3 (-20.4 to -2.2)) remained significantly correlated with expressive communication scaled score.

3.1.3. Outcome at two years

3.1.3.1. Neurological outcome: Cerebral palsy according to Gross Motor Function Classification System (GMFCS)

Ten children had CP according to the GMFSC. Two children had level 4, 7 children level 2 and one child level 3. One child (24 weeks GA at birth) with level 4 had diffuse cystic PVL, small CC, cerebellar haemorrhage, abnormal PLIC and dilated ventricles on MRI. The other child (25 weeks GA at birth) with level 4 had GMH-IVH, small CC and DEHSI on MRI. Level 3 was found in a child (24 weeks GA at birth) with very complicated neonatal respiratory course, which ended up in tracheostomy. On MRI dilated ventricles, increased extracerebral space and small CC were found, however no cystic lesions. Of 7 the children with level two GMFCS, two had unilateral HPI, one posthaemorrhagic ventricular dilatation requiring a shunt, 3 children had PWML and dilated ventricles with irregular ventricular borders and one infant showed only increased extracerebral space. CP did not correlate with GA at birth, birth weight, oxygen requirement at 36 weeks, postnatal hydrocortisone use, chorioamnionitis, chorioamnionitis with funisitis (p=0.06), and inotropic use. CP correlated on univariate anaylsis with IVH (p<0.01, r=0.38), ventricular dilatation (p=0.02, r=0.27),

small CC (p=0.02, r=0.27). There was a trend for HPI to correlate with CP (p=0.09). On multiple linear regression IVH remained a significant correlate (p=0.02, regression coefficient (95%CI) -0.3 (-0.5 to -0.08)).

3.1.3.2. Neurodevelopmental outcome

54/80 (68%) had follow-up (FU) assessment at two year corrected gestational age. 10 families moved abroad and 16 families did not attend any appointment despite several phone calls and written invitations. Table 3.8 shows the differences between the infants who had FU at one year CGA and those who did not have 2-years assessment. As CLD, inotropic use and BW are significantly related to gestational age at birth, it is not unexpected that the younger infants who had better FU attendance were smaller, had more often PDA, CLD and GMH-IVH.

	FU (n=54)	**No FU (n=26)**
Gestational age at birth (in weeks)	26.4 (2.3)	29.4 (2.7)
Birth weight (in grams)	907 (292)	1218 (468)
Gender (female (%))	60%	44%
Inotropic use	42%	30%
CLD	65%	44%
PDA	64%	40%
NEC	21%	11%
GMH-IVH	25%	7%
Cystic WM lesions	31%	4%
DEHSI	79%	78%
PWML	33%	33%

Table 3.8. Neonatal and imaging characteristic differences between infants who did attend and those who did not attend FU at two years corrected gestational age.

Table 3.9 shows the mean (SD) cognitive, motor and language scores of all preterm infants at two years corrected gestational age. There was no correlation between cognitive, motor or language outcome with maternal education.

	All preterm infants (n=54)	**Range**
Composite Cognitive Score	91.9 (13.3)	55 to 115
Composite Motor Score	90.4 (17.8)	49 to 124
Composite Language Score	92.3 (15.6)	55 to 135
Receptive Communication Scaled Score	9.0 (3.1)	1-17
Expressive Communication Scaled Score	8.2 (3.1)	2-15
Gross Motor Scaled Score	7.9 (2.8)	1-14
Fine Motor Scaled Score	9.3 (3.0)	1-16

Table 3.9. Mean (SD) cognitive, motor and language scores

Composite cognitive scores correlated with composite motor scores (Fig3.3) and there was a positive correlation between composite language and composite cognitive scores.

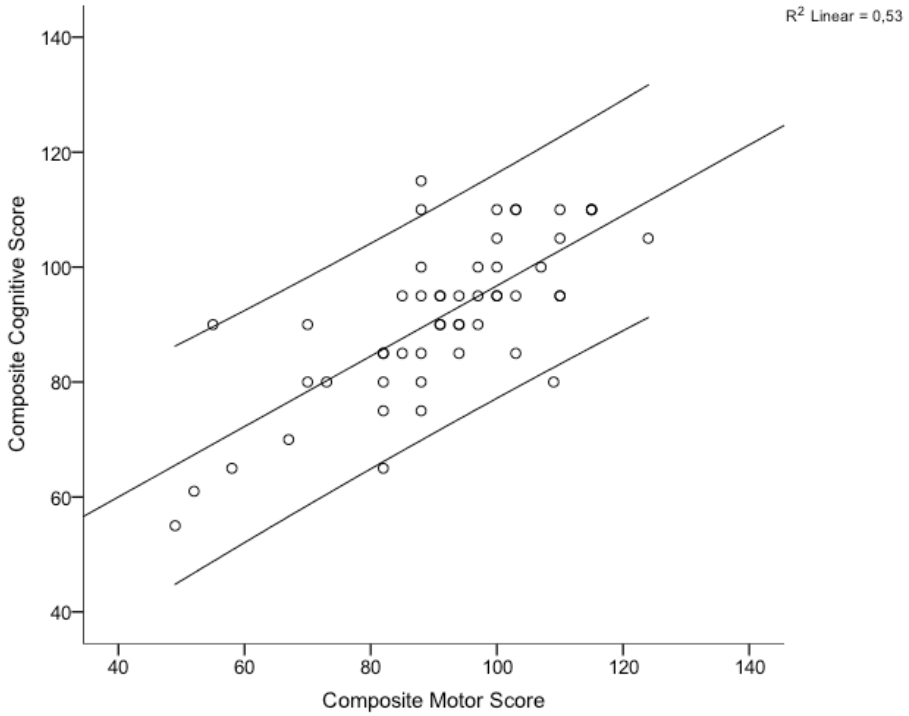


Fig.3.3. Scatterplot showing linear correlation between composite cognitive and motor scores at two years corrected gestational age. Inner line represents the linear fit, outer lines CI 95%

Composite cognitive scores correlated with receptive communication scaled scores and with expressive communication scaled scores as did fine with gross motor scaled scores.

3.1.3.3. Correlation of two years outcome with clinical data

Composite cognitive score was associated with birth weight, race groups, with younger and Caucasian infants having lower cognitive scores. On multiple linear regression only birth weight remained significantly correlated with composite cognitive scores.

Composite motor score correlated on univariate analysis with inotropic use and birth weight. No correlation was found with gender, gestational age at birth, race, postnatal hydrocortisone administration, NEC, PDA, oxygen requirement at 28 days of age and at 36 weeks corrected gestational age and chorioamnionitis with or without funisitis. Birth weight was the only significant correlate with gross motor scaled score.

Fine motor scaled scores correlated on univariate analysis with birth weight, race and inotropes. No correlation was found with gender, gestational age at birth, race, postnatal hydrocortisone administration, NEC, PDA, oxygen requirement at 28 days of age and at 36 weeks corrected gestational age and chorioamnionitis with or

without funisitis. Postnatal hydrocortisone administration remained the single significant correlate with fine motor scaled score on multiple linear regression.

Composite language scores correlated with birth weight and postnatal hydrocortisone administration on univariate analysis. No correlation was found with gender, gestational age at birth, race, postnatal hydrocortisone administration, NEC, PDA, oxygen requirement at 28 days of age and at 36 weeks corrected gestational age and chorioamnionitis with or without funisitis. Receptive communication scaled scores correlated with birth weight, gestational age at birth, gender and postnatal hydrocortisone administration. On multiple linear regression postnatal hydrocortisone administration and gender remained significantly correlated with receptive communication scaled score: female preterm infants having higher scores than male infants (9.9 vs 7.75). No clinical correlate could be found for expressive communication scaled score.

3.2. Comparison between one and two years outcome

Table 3.10 shows the differences in outcome scores between one and two years of age (paired sample testing). Cognitive scores were significantly higher at one year than at two years. Scaled gross motor scores were lower at two years whereas fine motor scaled scores were higher at two years. There were no significant differences in language composite and subscales scores or in composite motor scores between one and two years of age.

Mean (SD)	**At one year**	**At two years**	***p values***
Composite cognitive score	99.1 (13.9)	91.1 (13.4)	*<0.01*
Composite Motor score	89.5 (13.5)	91.3 (15.5)	*0.4*
Composite Language score	92.2 (12.0)	89.9 (18.4)	*0.3*
Receptive Communication Scaled Score	8.7 (2.5)	8.7 (3.5)	*1.0*
Expressive Communication Scaled Score	8.6 (2.6)	8.0 (3.2)	*0.3*
Gross Motor Scaled Score	9.0 (2.3)	7.6 (2.8)	*<0.01*
Fine Motor Scaled Score	7.1 (9.1)	9.1 (3.0)	*<0.01*

Table 3.10. Paired sample testing showing significant differences in composite cognitive and gross/ fine motor scaled scores between one and two year cognitive scores

The correlations between one and two year outcome are shown in the following scatterplots (Fig.3.4a-c)

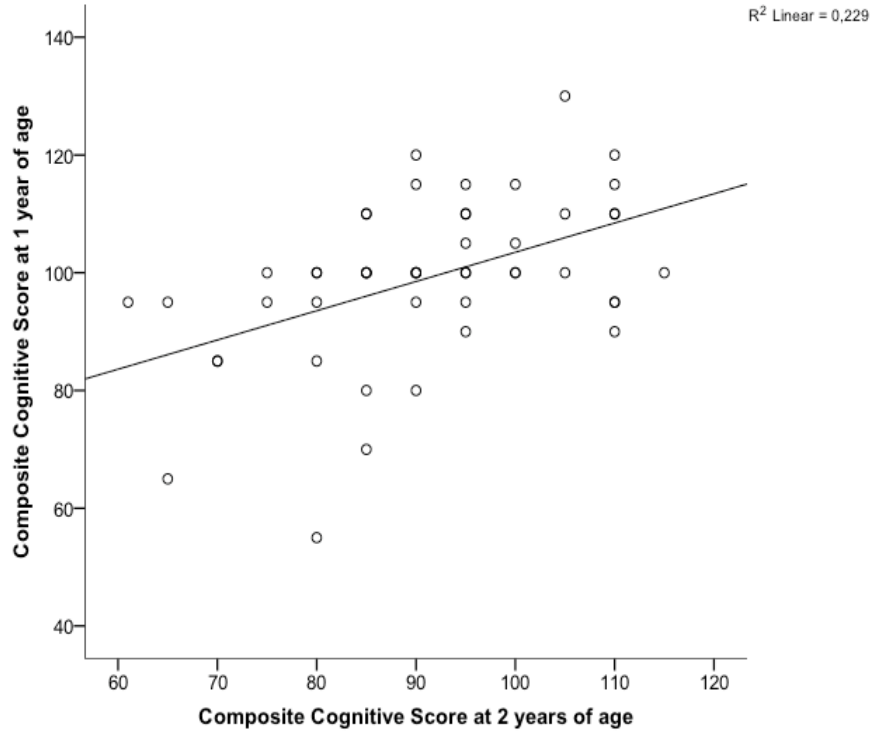


Fig.3.4a. Scatterplot showing correlation between one and two year composite cognitive scores

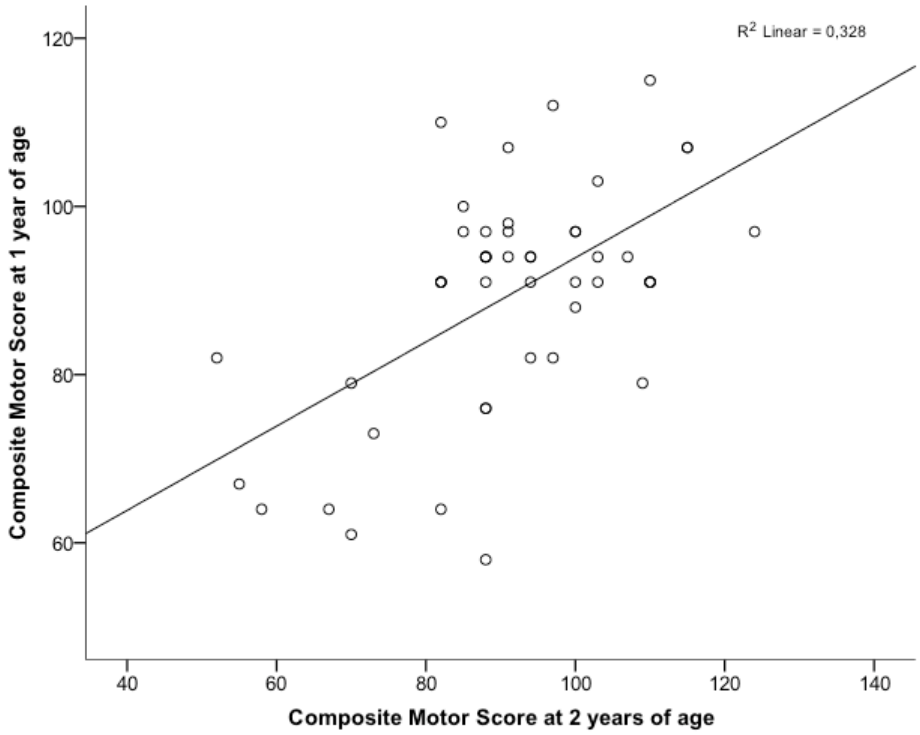


Fig.3.4b. Scatterplot showing correlation between one and two year composite motor scores

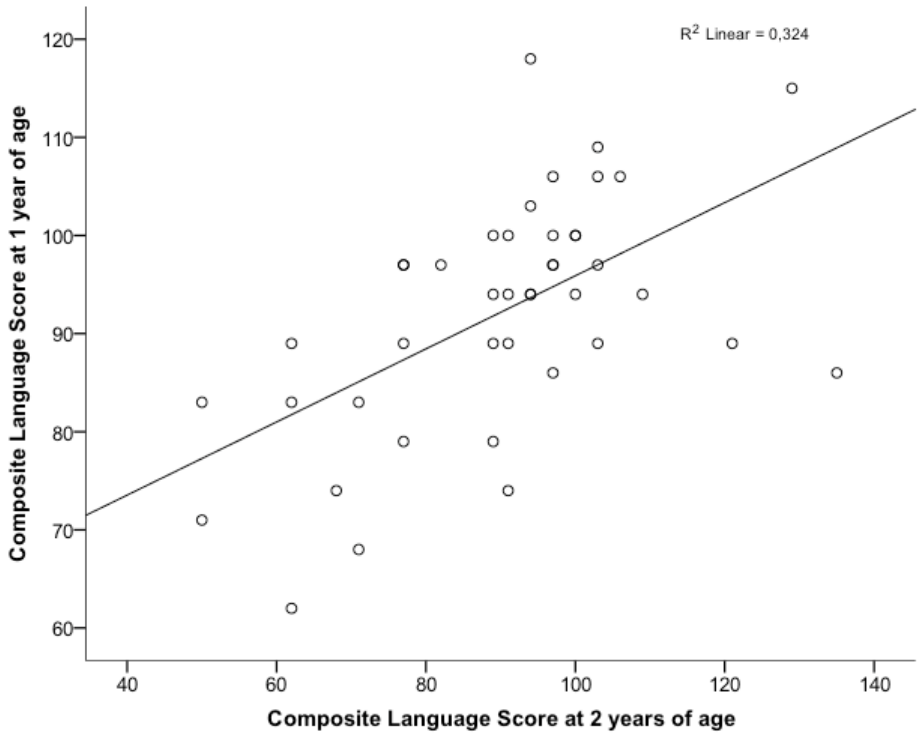


Fig.3.4c. Scatterplot showing correlation between one and two year composite language scores

3.3. Conventional MR

Axial T2 weighted images could be assessed in 78 infants; in two infants too many artefacts were present to accurately evaluate the findings. 3D FLASH images could be assessed in all 80 infants. Mean age at scanning was 40 weeks ranging between 37 and 44 weeks of corrected gestational age.

3.3.1. Germinal matrix haemorrhage (GMH) and intraventricluar haemorrhage (IVH)

At term equivalent age GMH was present in 14 (18%) infants: seven infants had unilateral GMH (five had right, two left) and 7 bilateral GMH. 16 (20%) infants had IVH with 2 unilateral IVH (right) and 14 bilateral IVH (Fig.3.5). Haemorrhage was isolated to the germinal matrix in eight infants, one infant had left sided GMH and bilateral IVH, one infant had right-sided GMH and IVH and two infants had right-sided GMH with bilateral IVH. Two infants had bilateral GMH-IVH (Table 3.11).

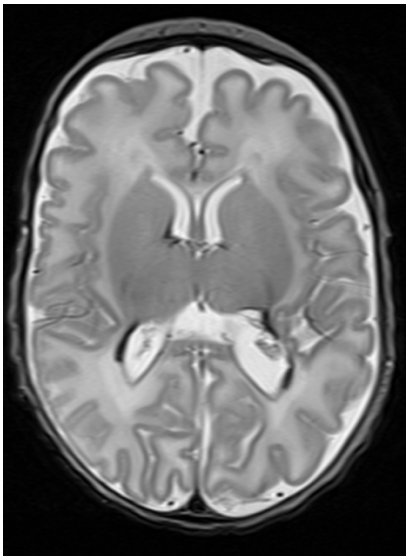


Fig.3.5. Axial T2 weighted MR image showing bilateral IVH

GMH-IVH		**IVH**			*Total*
		Right	Bilateral	None	
GMH	Right	1	2	2	5
	Left	0	1	1	2
	Bilateral	0	2	5	7
	None	1	9	56	66
	Total	2	14	64	80

Table 3.11. Localisation of GMH and IVH

Preterm infants with GMH had significantly lower mean GA (25.87 vs. 27.7 weeks, $p<0.01$) and BW (734.36 vs. 1066 grams, $p<0.01$) than preterm infants without GMH. The same significant correlation existed between the presence of IVH and GA ($p=0.02$) and BW ($p=0.045$). Presence of IVH correlated with the occurrence of

ventricular dilatation even when adjusted for GA and BW ($p<0.01$, regression coefficient (95%CI) -0.35 (-0.52 to -0.19). There was a significant association between IVH and cerebellar haemorrhage. GMH-IVH correlated with cystic PVL ($p=0.02$) and haemorrhagic infarction ($p<0.01$) on univariate analysis, however when corrected for GA then only haemorrhagic infarction remained significant ($p<0.01$).

3.3.2. Cerebellar haemorrhages

Cerebellar haemorrhagic lesions were present in 11 (14%) preterm infants. Three of the cerebellar haemorrhages were unilateral and eight were bilateral (Fig.3.6a-c). There was a significant correlation between the presence of GMH-IVH and cerebellar haemorrhage (*p<0.01*, regression coefficient (95%CI) -3.2. (-0.50 to 0.14)) even after correction for GA and BW. There was no correlation between cerebellar haemorrhage and PWML. The size of the cerebellum was assessed small in four infants; there was no correlation between size of cerebellum and the presence of haemorrhage. SI was increased in the cerebellar WM in 48 (60%) infants and in cerebellar grey matter in 34 (43%). There was a significant association between cerebellar haemorrhage and increased SI in the cerebellar grey matter (*p=0.029*) but not for cerebellar WM (*p=0.36*). Delayed myelination in the right and left PLIC was significantly associated with cerebellar haemorrhage.

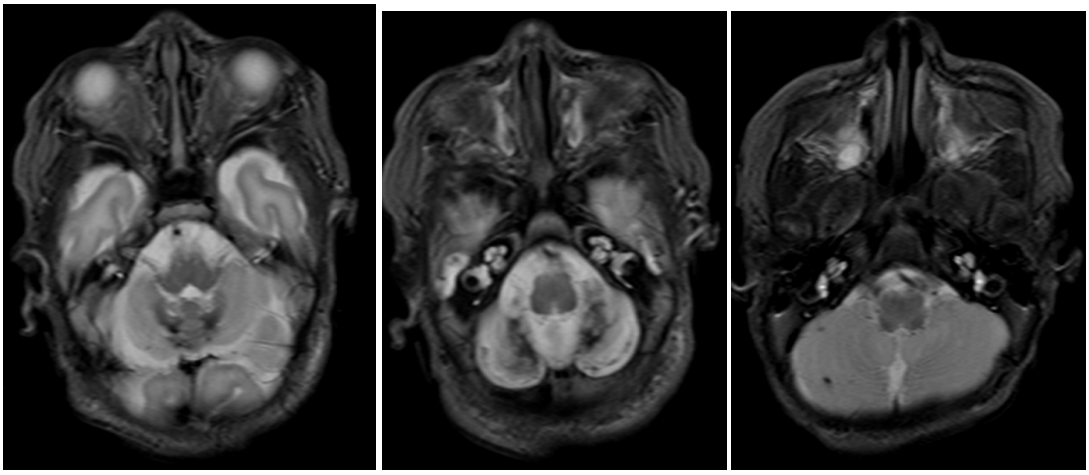


Fig.3.6a and b. Axial T2 weighted images showing bilateral cerebellar haemorrhages; c. axial T2 weighted image showing punctate haemorrhage in the right hemisphere

3.3.3. Extracerebral haemorrhages

Three (4%) infants had extracerebral haemorrhage: 2 infants showed subdural haemorrhage and one infant had an epidural haemorrhage. One infant with subdural haemorrhage had GMH and IVH and the infant with the epidural haemorrhage had GMH.

3.3.4. White matter lesions

3.3.4.1. Punctate white matter lesions (PWML)

26 (32.5%) infants showed punctate white matter lesions. Most of the lesions were located in the frontal and parietal lobe (Table 3.12). Five infants had lesions within the optical tract, three bilateral and two unilateral. CST was involved in seven infants with bilateral involvement in six (Fig.3.7a-c) and unilateral involvement in one infant.

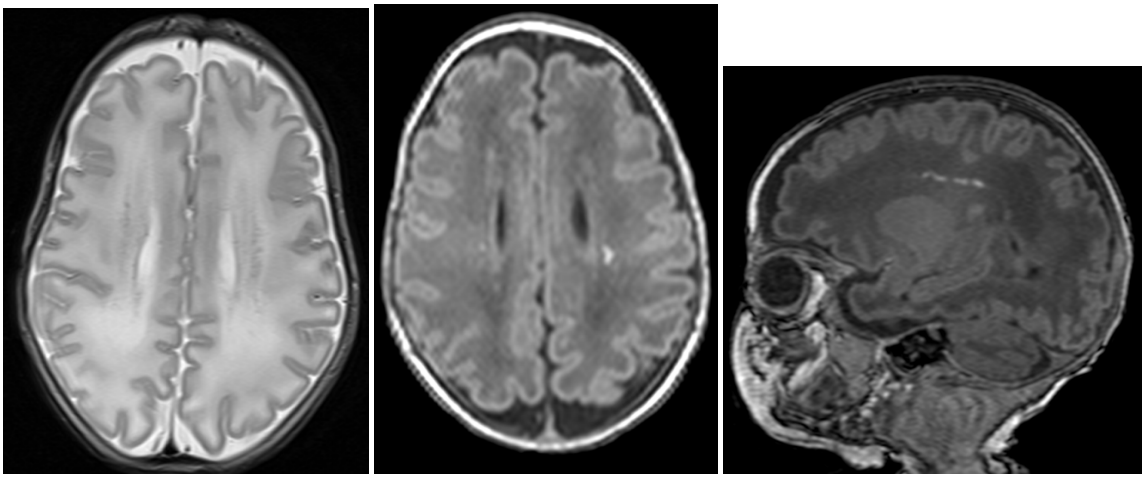


Fig.3.7a-c. PWML on T2 weighted, T1 weighted and sagittal T1 weighted images

PWML	PvWM right	PvWM left	DWM right	DWM left	CST right	CST left	OR right	OR left
Frontal lobe	4	4	5	4	0	0	0	1
Fronto-parietal lobe	3	3	1	2	2	2	0	0
Parietal lobe	3	3	4	2	1	0	0	0
Parieto-occipital lobe	3	2	1	1	0	0	0	0
Fronto-occipital lobe	0	0	1	1	0	0	0	0
Occipital lobe	0	0	0	0	0	0	1	2
All lobes	1	1	1	1	1	1	1	1

Table.3.12. Regional distribution of PWML within the WM

The minority of infants with PWML had more than 10 PWML; most infants had between 1-5 lesions, some between 6-9. Most lesions were smaller than 2mm in size. There was no significant correlation between PWML and the occurrence of GMH, IVH or cerebellar haemorrhages. There was a significant correlation between cystic white matter lesions and PWML, even after adjustment for GA and BW (*p=0.011*, regression coefficient (95%CI) -0.03(-0.56 to -0.077). The size of the corpus callosum correlated significantly with PWML ((*p=0.016*, regression coefficient (95%CI) -0.29(-0.54 to -0.056)) on univariate analysis. The presence of PWML correlated significantly with delayed myelination in the right PLIC on univariate

analyses (*p=0.02*), however after correction for cystic white matter lesions, the correlation became insignificant (*p=0.16*).

3.3.4.2. Diffuse excessive high signal intensity (DEHSI)

DEHSI was seen within cerebral WM on axial T2 weighted images in 62 (77.5%) of infants (Fig.3.8a and b). Cerebral WM DEHSI was associated with increased signal intensity in the cerebellar WM ($p<0.01$). There was no significant difference in GA and BW between infants with and without DEHSI. There was no association between DEHSI and GMH, IVH, cerebellar haemorrhage, PWML or cystic white matter lesions. Ventricular dilatation was associated with DEHSI (Fig.3.9). 32/35 infants with ventricular dilatation had DEHSI on imaging, even after adjusting for infants with cystic WM lesions. However, DEHSI was not associated with irregular ventricular borders or with enlarged ECS. 21/22 infants with small CC had DEHSI *(p=0.018).*

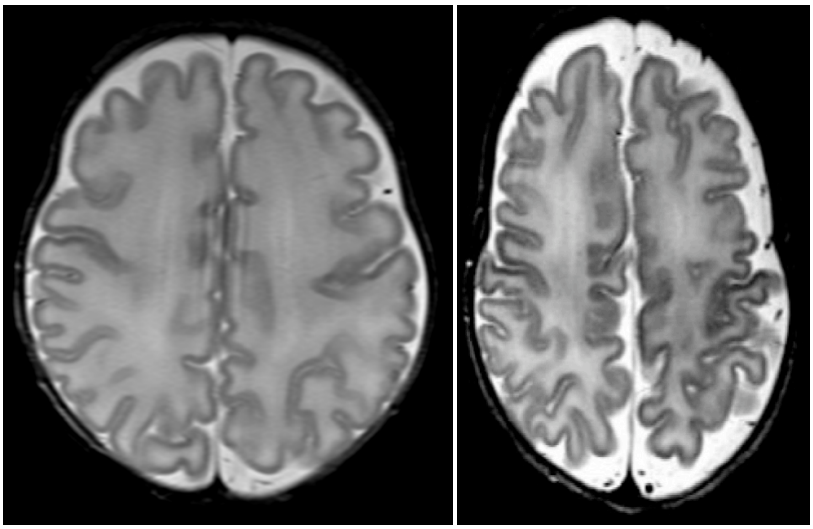


Fig.3.8a and b. Axial T2 weighted images showing normal signal intensity in a. and in b. increased signal intensity in the WM.

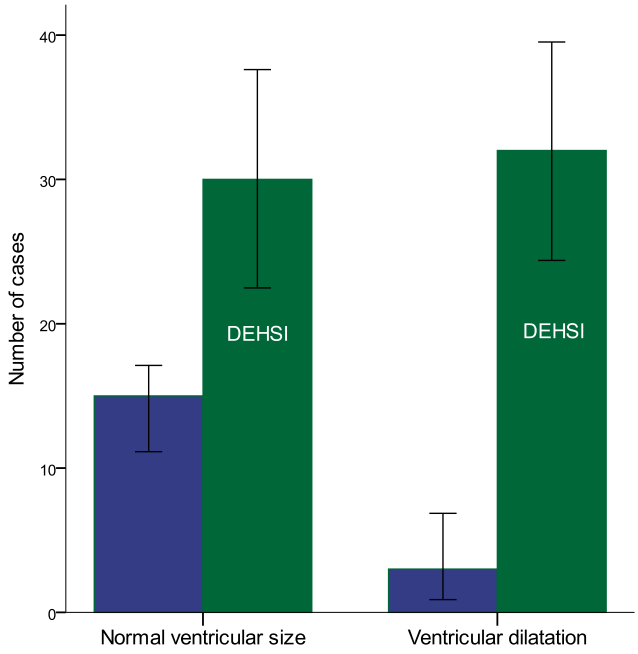


Fig.3.9. Bar chart showing the association between ventricular size and DEHSI. Dilated ventricles were associated with DEHSI. Error bars 95% CI

3.3.4.3. Cystic white matter lesions

3.3.4.3.1. Haemorrhagic parenchymal infarction (HPI)

Seven (9%) infants experienced HPI, all unilateral: 6 left sided and one right sided (Fig.3.10a and b). In one infant the cyst was frontally, in two infants it was in the parietal lobe and in four infants in the temporal lobe. PLIC was assessed for asymmetry and abnormal myelination (Fig. 3.11.a-c). All had delayed myelination in right or left PLIC, two infants had bilaterally delayed myelination in the PLIC. Asymmetrical PLIC was noted in 4 infants. HPI was significantly associated with GMH and IVH. There was no significant difference in BW and GA between infants with HPI and without HPI. On univariate analyses cerebellar haemorrhage was significantly associated with HPI (*p=0.01*, regression coefficient (CI) -0.44 (-0.78 to -0.108)), however on multiple linear regression this correlation became insignificant. There was no association between HPI and DEHSI, PWML or ventricular dilatation.

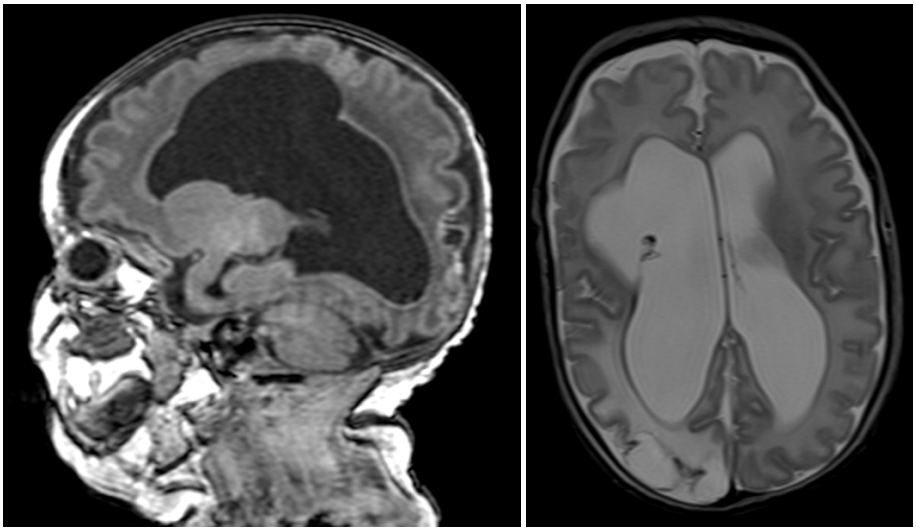


Fig.3.10a and b. showing unilateral haemorrhagic parenchymal infarction. In addition, in this patient there are also cystic changes within the temporal and occipital lobes.

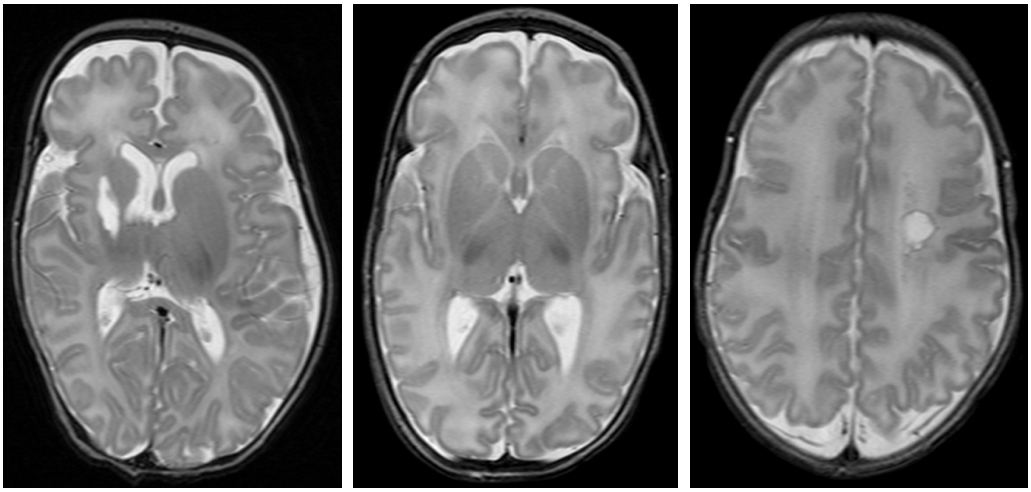


Fig.3.11. Axial T2 weighted images showing in a. right porencephalic cyst with asymmetrical PLIC, in b. symmetrical PLIC and in c. small porencephalic cyst within the central part of the CSO, same patient as in b.

3.3.4.3.2. Cystic periventricular leukomalacia (cPVL)

Cystic periventricular leukomalacia was noted in 11 (14%) preterm infants. The location of the cysts is shown in table 3.13. Most cysts were in the periventricular WM of the frontal and occipital lobe. In a few infants all lobes were affected. Most cysts were bilateral and were smaller than 2mm. Three infants (3.8%) had “classic” diffuse cPVL involving the parieto-occipital WM bilaterally in one case (Fig.3.12a), both fronto-parietal lobes in the second case and in the last case both occipital lobes; in two cases mainly the periventricular WM was affected, in the worst case periventricular WM, subcortical WM, the corticospinal tract and the optic radiation were involved. All other eight cases had focal cysts: in four cases the cysts were

unilateral and in 4 cases each the periventricular WM (Fig. 3.12b) and the deep WM were affected respectively.

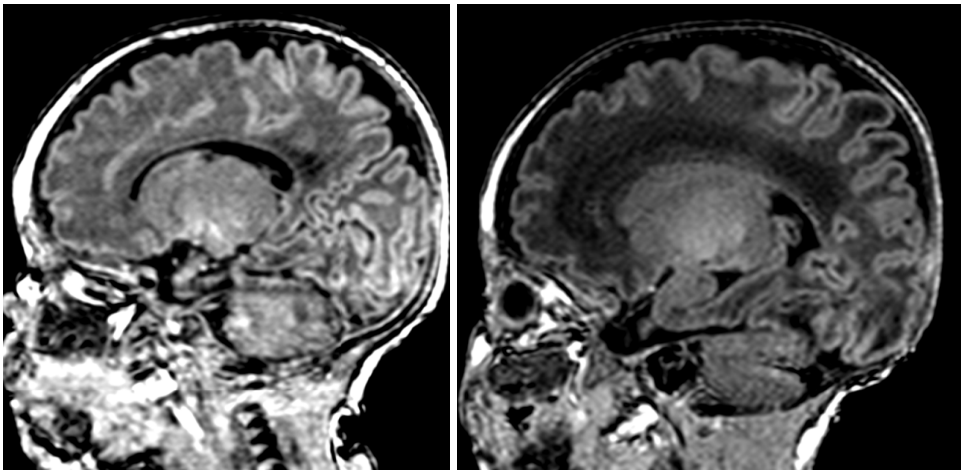


Fig.3.12.a. sagittal T1 weighted image showing cysts in the parito-occipital WM, and b. sagittal T1 weighted image showing focal cystic changes in frontal and occipital periventricular WM

Cystic WM lesions	PvWM right	PvWM left	DWM right	DWM left	CST right	CST left	OR right	OR left
Frontal lobe	3	2	2	2	0	0	0	0
Fronto-parietal lobe	0	0	1	1	0	0	0	0
Parietal lobe	0	0	0	0	0	0	0	0
Parieto-occipital lobe	1	1	0	0	0	0	0	0
Fronto-occipital lobe	0	0	0	1	0	0	0	0
Occipital lobe	2	1	0	1	0	0	0	0
All lobes	1	1	0	0	1	1	1	1

Table.3.13. Regional distribution of cystic PVL lesions within the WM

There was no significant difference in GA and BW between infants with and without cystic PVL. 9/11 infants with cystic PVL had DEHSI on T2 weighted images. There was a significant association between cystic PVL and irregular ventricular borders (*p<0.01*), ventricular dilatation *(p<0.01)* and GMH-IVH *(p=0.032).* A trend for association between PWML and cystic PVL *(p=0.057)* was noted. Delayed myelination in the left and right PLIC correlated with the presence of cystic PVL (*p= 0.01* and *p= 0.045* respectively). Myelination in the brainstem was delayed in infants with cystic PVL whereas there were no significant differences in myelination in the basal ganglia/ thalami between infants with and without cystic PVL.

3.3.5. Corpus callosum (CC)

Twenty (25%) infants had a small CC assessed on mid-sagittal T1 weighted images (Fig.3.13a and b). There was a significant correlation between small CC and HPI

(*p<0.01*), but not between small CC and cystic PVL (*p=0.15*). Further significant correlation was found between small CC and GMH-IVH and between small CC and delayed myelination of the right and left PLIC. On univariate analysis small CC correlated with birth weight, GA at birth and corrected age at MRI. On multiple linear regression birth weight was the only clinical variable that remained significantly associated with small corpus callosum.

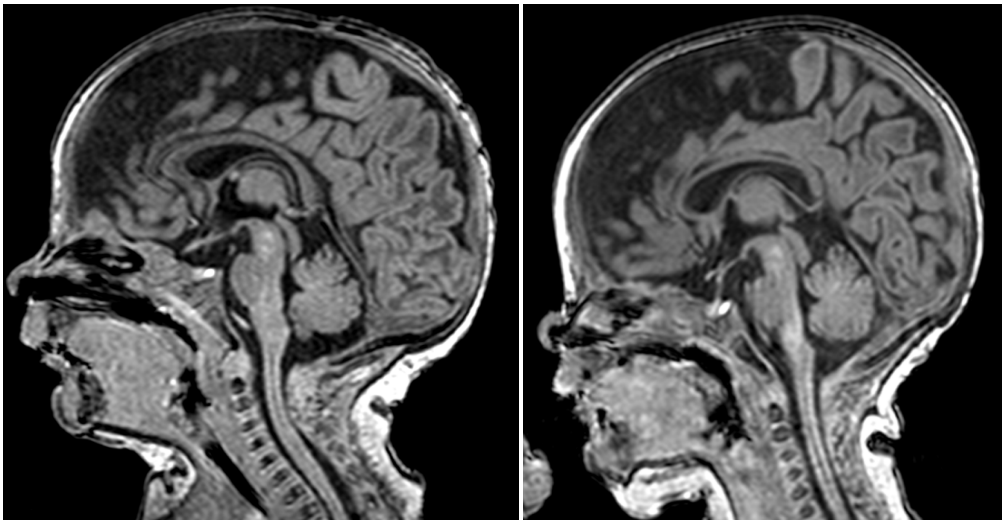


Fig.3.13a and b. T1 weighted midline sagittal MR image showing normal sized CC and b. small sized CC

3.3.6. Myelination

Myelination was assessed in the corticospinal tracts, the brainstem, the ventrolateral thalami and the putamina. Delay was defined as two weeks more immature than the corrected gestational age when scanning was done. Seven infants had delayed myelination in the left PLIC and nine in the right PLIC. Inferior to the PLIC the myelination was delayed in 6 infants bilaterally. When myelination was assessed superior to the PLIC it was delayed in five infants on the right side, and in six infants on the left side. Two infants had delayed myelination in the right and left ventrolateral thalamus, two infants in the left putamen and one in the right putamen. On univariate analysis irregular ventricular borders and ventricular dilatation were associated with delayed myelination in the right and left PLIC (*p=0.03 and p= 0.019*). The presence of any haemorrhage (GMH and/or IVH) was associated with delayed myelination in PLIC even when corrected for gestational age (*p<0.01*). Myelination in the brainstem was delayed in infants with cystic PVL whereas there were no significant differences in myelination in the basal ganglia/ thalami between infants with and without cystic PVL. Delayed myelination in the right and left PLIC was significantly associated with cerebellar haemorrhage.

3.3.7. Ventricular size

Thirty-five infants (44%) had ventricular dilatation and 20 infants (25%) had irregular ventricular borders. Ventricular dilatation was associated with the presence of IVH even when corrected for gestational age (*p<0.01*), not with the presence of GMH. Ventricular dilatation was associated with irregular borders. The association between ventricular size and myelination/DEHSI was discussed above. Ventricular dilatation was significantly associated with the presence of cystic PVL on univariate and multiple linear regression (*p=0.016*). Haemorrhagic infarction was not associated with ventricular dilatation.

3.3.8. Cerebellar size

In four infants the size of the cerebellum was assessed as small. On univariate analysis there was a significant correlation between cerebellar size and cerebellar haemorrhage (*p=0.03*), the correlation remaining significant after correction for gestational age.

3.3.9. Deep grey matter

3.3.9.1. Caudate nuclei

In fourteen infants the right caudate was abnormal with haemorrhagic lesions in 2 infants, cystic lesions in seven infants, in three infants cystic and haemorrhagic lesions and in two infants the caudate was assessed as small in size. Seven lesions were found in the left caudate area with two infants having cystic and haemorrhagic lesions, one infant having haemorrhagic and four infants having cystic lesions alone.

3.3.9.2. Putamina

A haemorrhagic and a cystic lesion each were found in the right putamen; in the left putamen one haemorrhagic lesion was seen.

3.3.9.3. Thalami

In four infants a haemorrhagic lesion was seen in the right thalamus and in one infant in the left thalamus; cystic lesions were seen in two infants bilaterally.

3.3.9.4. Globus pallidus

Haemorrhagic lesions were seen in two infants.

3.4. Correlation of MR findings with clinical data

3.4.1. Haemorrhages

On univariate analysis GMH-IVH was associated with gestational age at birth, birth weight, PDA and with inotropic use. Pneumothorax was not associated with the presence of GMH-IVH. On multiple linear regression, only inotropic use remained significantly correlated with the presence or GMH-IVH (*p=0.02*, regression coefficient (95%CI) -0.2 (-0.40 to -0.041)). No association could be found between GMH-IVH and chorioamnionitis with or without funisitis.

On univariate and multiple linear regression the presence of GMH and/or IVH was associated with cerebellar haemorrhages. Neither inotropic use nor PDA was significantly associated with cerebellar haemorrhages *(p=0.055 and p=0.26 respectively).* Chorioamnionitis or chorioamnionitis with funisitis was not associated with cerebellar haemorrhages.

3.4.2. PWML

PWML was not associated with any clinical data.

3.4.3. DEHSI

There was no correlation between DEHSI and any clinical data such as gestational age, birth weight, CLD, inotropic use, chorioamnionitis with funisitis, NEC, PDA and others.

3.4.4. Cystic WM lesions

Cystic WM lesions were not associated with gestational age at birth, birth weight, APGAR at 5', worst BE/lactate within first 24 hours, highest CRP within first 3 days (*p=0.07*), CLD, inotropic use (*p=0.07*), hydrocortisone use, chorioamnionitis with funisitis, NEC, PDA or any other clinical parameters. When cPVL and HPI were analysed separately, inotropic use correlated with the presence of HPI on multiple linear regression (*p=0.046*). cPVL correlated significantly with worst pH within first 24 hours, although not with worst lactate or worst BE. pCO_2 was not recorded in the minimal dataset. If only the three classic cPVL cases were analysed, then a correlation with the presence with chorioamnionitis and the worst pH within the first 24 hours was highly significant even after correction for PDA, NEC, CLD and inotropic use.

3.4.5. Size of corpus callosum

On univariate analysis the size of the corpus callosum was associated with gestational age, birth weight and inotropic use; no correlation was found with chorioamnionitis with funisitis, NEC, PDA, hydrocortisone use and CLD. On multiple linear regression inotropes remained significantly related to small corpus callosum.

3.5. Correlation of MR findings with neurodevelopmental outcome

3.5.1. At one year

3.5.1.1. T tests and linear regression

3.5.1.1.1. GMH-IVH

Significant differences in cognitive, motor and language outcome were found between infants with GMH-IVH compared to those without GMH-IVH (only infants without cystic WM lesions were included in analysis) (Table 3.14). The differences remained significant after correcting for gestational age and birth weight.

	PT no GMH-IVH (n=40)	**PT with GMH-IVH (n=14)**	***p value***
Composite Cognitive Score	92.6 (14.8)	86.4 (10.0)	*0.09*
Composite Motor Score	94.1 (15.6)	84.0 (16.5)	*0.05*
Composite Language Score	93.6 (16.6)	79.3 (17.8)	*0.03*
Receptive Communication Scaled Score	9.6 (2.9)	6.5 (3.1)	*<0.01*
Expressive Communication Scaled Score	8.8 (2.8)	6.5 (3.1)	*0.02*
Gross Motor Scaled Score	8.1 (2.8)	7.0 (3.2)	*0.3*
Fine Motor Scaled Score	7.6 (2.3)	5.3 (3.5)	*0.03*

Table 3.14. Cognitive, motor and language outcome in preterm infants with and without GMH-IVH

The presence of GMH correlated well with composite cognitive score on univariate analysis, but if adjusted for gestational age, birth weight and cystic WM lesions, the correlation became insignificant. The correlation between IVH and composite cognitive score remained significant after adjustment for gestational age at birth, birth weight and cystic WM lesions. Composite motor scores were associated with IVH but not with GMH on multiple (adjustment for gestational age, birth weight, cystic WM lesions). Neither GMH nor IVH were associated with composite language scores on multiple linear regression

3.5.1.1.2. Cerebellar haemorrhages

8/61 preterm infants had cerebellar haemorrhages: these correlated significantly with cognitive, motor and language outcome at one year. Cerebellar haemorrhage was associated with GMH-IVH, gestational age and birth weight. Multiple linear regression was done to see whether the difference between cerebellar haemorrhages and cognitive, motor or language outcome at one year remained significant after correction for GA, BW and GMH-IVH (Table 3.15).

Cerebellar haemorrhages	***p value***	**Regression coefficient (95% CI)**
Composite cognitive score	0.02	11.46 (2.0 to 20.9)
Composite language score	<0.01	12.9 (3.6 to 22.2)
Receptive Communication Scaled Score	0.03	2.4 (0.3 to 4.5)

Expressive Communication Scaled Score	0.04	2.1 (0.09 to 4.1)
Composite motor score	0.05	9.9 (0.001 to 19.9)
Gross Motor Scaled Score	0.2	1.1 (-0.7 to 2.9)
Fine Motor Scaled Score	0.05	2.1 (-0.01 to 4.3)

Table 3.15. Multiple linear regression with correction for GA, BW and GMH-IVH showing significant correlation with cognitive and language scores

3.5.1.1.3. DEHSI

After preterm infants with cystic lesions were excluded, outcome measures were compared between preterm infants with DEHSI and without DEHSI (Table 3.16). Table 3.16 shows the differences revealing similar cognitive and language scores. Significantly higher composite motor scores were found in preterm infants without DEHSI compared with those with DEHSI. When fine and gross motor function were assessed, fine motor scores were contributing significantly to the motor score difference between preterm infants with and without DEHSI, even after correction for gestational age (*p=0.036,* regression coefficient (95%CI) 8.9 (0.63 to 17.3)).

	PT no DEHSI (n=11)	**PT with DEHSI (n=35)**	***p value***
Composite Cognitive Score	107.7 (10.8)	101.0 (12.9)	*0.10*
Composite Motor Score	99.1 (10.4)	89.8 (12.8)	*0.02*
Composite Language Score	98.3 (15.3)	90.3 (11.8)	*0.14*
Receptive Communication Scaled Score	9.2 (3.9)	8.2 (2.4)	*0.47*
Expressive Communication Scaled Score	9.2 (1.6)	8.7 (2.7)	*0.50*
Gross Motor Scaled Score	9.9 (1.7)	9.32 (2.6)	*0.44*
Fine Motor Scaled Score	8.78 (0.9)	7.2 (2.6)	*<0.01*

Table 3.16. Cognitive, motor and language outcome in preterm infants with and without DEHSI after exclusion of preterm infants with cystic lesion

3.5.1.1.4. Cystic WM lesions

Table 3.17 shows the differences in cognitive, motor and language outcome measures in preterm infants with (n=11) and without cystic WM lesions (n=46.) Preterm infants with cystic WM lesions had significantly lower scores for cognitive, motor and language outcome. Expressive communication scaled scores were significantly higher in preterm infants without cystic WM lesions compared to preterm infants with cystic WM lesions, however no significant difference was found in receptive communication scores. Significantly higher scaled gross motor scores were found in preterm infants without cystic WM lesions compared to those with cystic WM lesions, even after correction for gestational age (*p=0.03,* regression coefficient (95%CI) 8.6 (0.8 to 16.1)).

	PT no WM cystic lesions (n=46)	PT with WM cystic lesions (n=11)	*p value*
Composite Cognitive Score	102.6 (12.6)	90.7 (15.5)	*0.01*
Composite Motor Score	92.0 (12.8)	82.7 (13.4)	*0.01*
Composite Language Score	92.3 (13.0)	83.3 (10.5)	*0.03*
Receptive Communication Scaled Score	8.4 (2.7)	7.4 (2.3)	*0.17*
Expressive Communication Scaled Score	8.8 (2.6)	6.6 (1.6)	*<0.01*
Gross Motor Scaled Score	9.4 (2.4)	7.7 (1.3)	*<0.01*
Fine Motor Scaled Score	7.5 (2.4)	5.9 (3.2)	*0.11*

Table 3.17. Cognitive, motor and language outcome in preterm infants with and without cystic lesions

Six infants with HPI when compared to preterm infants without any cystic lesions had significantly lower cognitive, motor and language scores. Gross and fine motor scores were associated with the presence of HPI. The correlation between HPI and expressive communication was significant (*p=0.03*) but not for receptive communication scaled scores (*p=0.09*).

3.5.1.1.5. Size of CC

19/61 infants had small corpus callosum on conventional MR. Infants with small corpus callosum had lower cognitive, motor and language scores compared to those with normal size corpus callosum (Table 3.18). The correlation remained significant after correction for birth weight.

	PT with normal CC (n=19)	PT with small CC (n=42)	*p value*
Composite Cognitive Score	104.5 (11.8)	90.00 (14.7)	*<0.01*
Composite Motor Score	94.0 (11.6)	81.6 (10.3)	*<0.01*
Composite Language Score	93.4 (12.5)	81.6 (10.4)	*<0.01*
Receptive Communication Scaled Score	8.7 (2.7)	6.9 (2.2)	*0.01*
Expressive Communication Scaled Score	8.9 (2.6)	6.8 (1.8)	*<0.01*
Gross Motor Scaled Score	9.6 (2.4)	7.8 (1.5)	*<0.01*
Fine Motor Scaled Score	7.9 (2.0)	5.6 (3.2)	*<0.01*

Table 3.18. Cognitive, motor and language outcome in preterm infants with normal size CC and small CC assessed visually on conventional MR

3.5.1.1.6. PWML

The presence of PWML correlated only with fine motor scaled scores.

3.5.1.2. Binary logistic regression and Odd ratios

3.5.1.2.1. Cognitive outcome

The infants were grouped into those who had cognitive scores below or above 85. Stepwise regression was performed with the following variables: GA, gender, birthweight, IVH, PWML, DEHSI, cerebellar haemorrhage, HPI, cPVL and small CC. HPI remained the only independent risk factor for cognitive scores below 85 at one year of age (Table 3.19).

Cognitive score < 85	***p values***	**Odds Ratio**	**95% CI**
HPI	*<0.01*	17.3	2.4 to 125.4

Table 3.19. HPI remained independent risk factors for cognitive outcome at one year

If cPVL and HPI were merged together as cystic WM lesions, then small CC remained the only independent risk factors, however with wide CI (Table 3.20).

Cognitive score <85	***p value***	**Odds Ratio**	**95% CI**
Small CC	*0.02*	14.6	1.6 to 136.3

Table 3.20. Small CC remained independent risk factors for cogntive outcome at one year

3.5.1.2.2. Motor outcome

The infants were grouped into those who had motor scores below or above 85. Stepwise regression was performed with the following variables: GA, gender, birthweight, IVH, PWML, DEHSI, cerebellar haemorrhage, HPI, cPVL and small CC. IVH remained the only significant risk factor for motor scores below 85 (Table 3.21).

Motor score < 85	***p value***	**Exp (B)**	**95% CI**
IVH	*<0.01*	10.6	2.4 to 46.7

Table 3.21. IVH remained the only independent risk factor for motor outcome at one year

If cPVL and HPI were merged together as cystic WM lesions, then small CC and IVH remained risk factors for motor scores below 85 (Table 3.22).

Motor score < 85	***p values***	**Exp (B)**	**95% CI**
IVH	*0.12*	6.4	1.4 to 27.4
Small CC	*<0.01*	7.4	1.8 to 29.0

Table 3.22. IVH and small CC remained independent risk factors for motor outcome at one year

3.5.1.2.3. Language outcome

Stepwise regression was performed with the following variables: GA, gender, birthweight, IVH, PWML, DEHSI, cerebellar haemorrhage, HPI, cPVL and small CC. Cerebellar haemorrhage, small CC and gender remained independent risk factors for

language outcome below 85. This correlation remained the same when HPI and cPVL were merged together as cystic WM lesions (Table 3.23). The CI for cerebellar haemorrhage was very wide.

Language score < 85	*p values*	Exp (B)	95% CI
Cerebellar haemorrhage	*<0.01*	23.3	2.4 to 220.81
Small CC	*<0.01*	8.2	1.8 to 36.0
Gender (male)	*0.05*	5.5	0.9 to 31.3

Table 3.23. Cerebellar haemorrhage, small CC and gender remained independent risk factors for motor outcome at one year

3.5.2. At two years

3.5.2.1. T tests and linear regression analysis

3.5.2.1.1. GMH-IVH

Significant differences were found in composite cognitive, motor and language scores between preterm infants with and those without GMH-IVH (Table 3.24). The differences remained significant after adjustment for gestational age at birth and birth weight.

	PT no GMH-IVH (n=33)	PT with GMH-IVH (n=12)	*p value*
Composite Cognitive Score	93.6 (13.7)	86.5 (10.5)	*0.06*
Composite Motor Score	95.3 (14.0)	83.7 (17.2)	*0.04*
Composite Language Score	94.2 (2.7)	79.3 (17.8)	*0.02*
Receptive Communication Scaled Score	9.7 (2.9)	6.8 (3.0)	*0.01*
Expressive Communication Scaled Score	8.9 (2.7)	6.0 (3.2)	*0.01*
Gross Motor Scaled Score	8.3 (2.6)	6.9 (3.3)	*0.2*
Fine Motor Scaled Score	9.9 (2.8)	7.6 (2.7)	*0.02*

Table 3.24. Cognitive, motor and language outcome in preterm infants with and without GMH-IVH

The presence of GMH correlated with composite motor scores and with fine motor scaled scores. No correlation was found between GMH and composite language scores. The expressive communication scaled score was related to the presence of GMH (*p=0.03*) and receptive communication scaled score (*p=0.05*).

The presence of IVH correlated with composite cognitive score (*p<0.01*) and composite motor score (*p=0.015*), fine and gross motor scaled score (*p=0.01* and

p<0.01 respectively). No correlation was found between IVH and any language outcome measure.

3.5.2.1.2. Cerebellar haemorrhages

After adjustment for gestational age at birth and the presence of GMH-IVH there was a significant correlation between composite cognitive score and cerebellar haemorrhages (*p=0.03*).

3.5.2.1.3. DEHSI

After preterm infants with cystic lesions were excluded, two years outcome measures were compared between preterm infants with DEHSI and without DEHSI. No significant differences were found in cognitive, motor or language scores between infants with DEHSI and those without DEHSI. On linear regression DEHSI was an independent risk factor for expressive language outcome at two years.

3.5.2.1.4. Cystic WM lesions (HPI and cPVL)

Table 3.25 shows the differences in composite cognitive, language and motor scores between preterm infants with and without cystic WM lesions. Preterm infants with cystic WM lesions had significantly lower scores for cognitive, motor, language outcome than preterm infants without cystic WM lesions, even after adjustment for gestational age and GMH-IVH.

Mean (SD)	**PT no cystic WM lesions (n=36)**	**PT with cystic WM lesions (n=16)**	***p values***
Composite Cognitive Score	95.9 (11.6)	82.6 (12.3)	*<0.01*
Composite Motor Score	95.9 (13.4)	84.4 (17.4)	*0.03*
Composite Language Score	94.8 (17.9)	80.2 (13.9)	*<0.01*
Receptive Communication Scaled Score	9.75 (3.2)	7.4 (2.4)	*0.01*
Expressive Communication Scaled Score	9.0 (3.0)	6.5 (2.7)	*<0.01*
Gross Motor Scaled Score	8.5 (2.5)	6.5 (3.1)	*0.04*
Fine Motor Scaled Score	9.9 (2.8)	7.8 (2.9)	*0.03*

Table 3.25. Cognitive, motor and language outcome in preterm infants with and without cystic WM lesions

Table 3.26 shows the differences in outcome in infants with and without HPI after exclusion of infants with cPVL. Infants with HPI had significantly lower scores in all outcome measures.

Mean (SD)	PT no HPI (n=37)	PT with HPI (n=6)	*p values*
Composite Cognitive Score	95.6 (11.6)	78.3 (9.3)	*<0.01*
Composite Motor Score	95.8 (13.3)	74.5 (12.4)	*0.01*
Composite Language Score	94.8 (17.9)	76.8 (12.4)	*0.01*
Receptive Communication Scaled Score	9.5 (3.4)	6.7 (1.9)	*0.01*
Expressive Communication Scaled Score	9.0 (3.0)	5.3 (2.5)	*0.01*
Gross Motor Scaled Score	8.5 (2.5)	4.7 (2.8)	*0.02*
Fine Motor Scaled Score	9.9 (2.8)	6.8 (2.7)	*0.04*

Table 3.26. Cognitive, motor and language outcome in preterm infants with and without HPI, after exclusion of the infants with cPVL

Composite motor scores ranged from 58 to 97. The infants with CP and HPI had motor scores of 71 and 68 respectively. The infant with composite motor score of 97 had a very small cystic lesion anterior to the corticospinal tract with symmetrical PLIC and was normal on neurological examination. Two infants with HPI and hemiplegia had asymmetrical PLIC; the third infant with asymmetrical PLIC had GMFSC of 2 but no asymmetry.

The gross motor scaled score correlated significantly with HPI ($p<0.01$) and fine motor scaled scores tended to correlate with HPI ($p=0.05$). After correction with IVH, the correlation remained significant between HPI and gross motor scaled scores ($p<0.01$). On univariate analysis expressive communication scaled scores correlated significantly with the presence of HPI ($p=0.02$) and there was a trend of correlation between receptive communication scaled score and HPI. After correction for IVH, the correlation between expressive scaled communication scores and HPI remained significant ($p=0.04$).

Table 3.27 shows the differences in outcome in infants with and without cPVL after exclusion of infants with HPI.

Mean (SD)	PT no cPVL (n=37)	PT with cPVL (n=11)	*p values*
Composite Cognitive Score	95.7 (11.6)	82.4 (15.7)	*0.02*
Composite Motor Score	95.7 (13.2)	86.6 (20.6)	*0.2*
Composite Language Score	94.7 (17.9)	81.4 (13.6)	*0.03*
Receptive Communication Scaled Score	9.5 (3.4)	7.6 (2.7)	*0.08*
Expressive Communication Scaled Score	9.0 (3.0)	7.0 (2.7)	*0.06*
Gross Motor Scaled Score	8.5 (2.5)	7.1 (3.2)	*0.3*
Fine Motor Scaled Score	9.9 (2.7)	7.8 (3.6)	*0.1*

Table 3.27. Cognitive, motor and language outcome in preterm infants with and without cPVL, after exclusion of the infants with HPI

There was no significant difference in outcome measures between infants with "classic PVL" and those without "classic PVL".

3.5.2.1.5. Size of CC

The correlation between small corpus callosum and cognitive, motor and language outcome was analysed in infants without cystic WM lesions (Table 3.28).

Small corpus callosum	*p value*	Regression coefficient (95% CI)
Composite cognitive score	0.14	-8.8 (-16.1 to 2.4)
Composite language score	0.25	-8.9 (-24.4 to 6.5)
Receptive Communication Scaled Score	0.3	-1.3 (-4.1 to1.5)
Expressive Communication Scaled Score	0.04	-2.6 (-5.1 to -0.9)
Composite motor score	<0.01	-16.8 (-26.9 to -6.8)
Gross Motor Scaled Score	<0.01	-3.2 (-5.1 to -1.3)
Fine Motor Scaled Score	<0.01	-3.0 (-5.1 to -0.9)

Table 3.28. Linear regression between small corpus callosum and cognitive, language and motor outcome in preterm infants without cystic WM lesions.

3.5.2.1.6. PWML

PWML correlated with composite motor score and fine motor scaled score in preterm infants without cystic WM lesions. Bilateral pvPWML correlated with motor outcome. No correlations were found between PWML and cognitive or language outcome

3.5.2.2. Binary logistic regression and odds ratios

3.5.2.2.1. Cognitive outcome (Tables 3.29 and 3.30)

The infants were grouped into those who had cognitive scores below or above 85. Stepwise regression was performed with the following variables: GA, gender, birthweight, IVH, PWML, DEHSI, cerebellar haemorrhage, HPI, cPVL and small CC. Independent risk factors for cognitive scores below 85 were IVH and cerebellar haemorrhages.

Cognitive score < 85	*p values*	Exp (B)	95% CI
IVH	*0.03*	5.1	1.2 to 22.1
Cerebellar haemorrhage	*0.03*	6.9	1.2 to 41.6

Table 3.29. Binary logistic regression showing risk factors for cognitive scores below 85 at 2 years

If cPVL and HPI were merged together as cystic WM lesions, IVH and cystic WM lesions remained independent risk factors for cognitive scores < 85.

Cognitive score < 85	*p values*	Exp (B)	95% CI
IVH	*0.013*	6.7	1.5 to 30.4
Cystic WM lesions	*0.004*	7.9	1.9 to 32.5

Table 3.30. Binary logistic regression showing risk factors for cognitive scores below 85 at 2 years, HPI and cPVL as WM lesions together

3.5.2.2.2. Motor outcome (Tables 3.31 and 3.32)

The infants were grouped into those who had motor scores below or above 85. Stepwise regression was performed with the following variables: GA, gender, birthweight, IVH, PWML, DEHSI, cerebellar haemorrhage, HPI, cPVL and small CC. PWML and HPI remained independent risk factors for motor scores below 85.

Motor score < 85	***p values***	**Exp (B)**	**95% CI**
PWML	*0.049*	4.2	1.0 to 16.9
HPI	*0.016*	17.8	1.7 to 184.7

Table 3.31. Binary logistic regression showing risk factors for motor scores below 85 at 2 years

When cPVL and HPI were merged together as cystic WM lesions, then small CC remained the only independent risk factor for motor scores below 85.

Motor score < 85	***p values***	**Exp (B)**	**95% CI**
Small CC	*0.005*	6.4	1.7 to 23.8

Table 3.32. Binary logistic regression showing risk factors for motor scores below 85 at 2 years, HPI and cPVL as WM lesions together

3.5.2.2.3. Language outcome (Table 3.33)

Stepwise regression was performed with the following variables: GA, gender, birthweight, IVH, PWML, DEHSI, cerebellar haemorrhage, HPI, cPVL and small CC. Birthweight and gender remained the only significant independent risk factors for motor outcome, hence smaller or male infants had worse language outcome than female or larger infants. This correlation remained the same when HPI and cPVL were merged together as cystic WM lesions.

Language score < 85	***p values***	**Exp (B)**	**95% CI**
Gender	*0.01*	0.9	0.98 to 0.99
Birthweight	*0.038*	5.8	1.0 to 30.6

Table 3.33. Binary logistic regression showing risk factors for language scores below 85 at 2 years

3.5.2.3. Linear regression for subscales

3.5.2.3.1.1. Gross motor and fine motor scaled scores

On linear regression after correction for GA at birth, cerebellar haemorrhages, IVH, HPI, cPVL and for PWML, small CC and BW remained independent risk factors for fine motor scaled scores at 2 years (Table 3.34). Small CC remained an independent risk factor for gross motor outcome.

Fine motor scaled scores	***p values***	**Regression coefficient**	**95% CI**
Small CC	*<0.01*	-2.4	-4.0 to -0.7
Birth weight	*0.02*	0.3	0.1 to 0.6
Gross motor scaled scores	***p values***	**Regression coefficient**	**95% CI**

Small CC	*<0.01*	-2.4	-4.4 to -1.4

Table 3.34. Linear regression showing risk factors for motor subscales at 2 years

3.5.2.3.1.2. Receptive and expressive communication language scaled scores

On linear regression after correction for BW, GA at birth, cerebellar haemorrhages, IVH, HPI, and for cPVL, BW remained an independent risk factor for receptive language scaled scores at 2 years and small CC and DEHSI for expressive language skills (Table 3.35).

Receptive language scaled scores	***p values***	**Regression coefficient**	**95% CI**
Birth weight	*0.03*	0.004	0.001 to 0.07
Expressive language scaled scores	***p values***	**Regression coefficient**	**95% CI**
Small CC	*<0.01*	-3.5	-5.3 to 1.8
DEHSI	*0.049*	2	0.01 to 4.1

Table 3.35. Linear regression showing risk factors for language subscales at 2 years

Summary:

Independent risk factors for cognitive outcome <85 were IVH, cerebellar haemorrhage and cystic WM lesions, for motor outcome <85 HPI, HPI and small CC, for language outcome <85 gender and birthweight and no MR variable. Motor subscales correlated with small CC and birth weight, whereas language subscales correlated with birth weight, small CC and DEHSI.

3.6. T2 measurements

3.6.1. T2 relaxometry

3.6.1.1. T2 with two different T2 relaxometry sequences

3.6.1.1.1. Phantom studies

Distilled water ADC at 22.9˚C was 2.155 ± 0.011 $x10^{-3}mm^2/sec$, which is comparable with published values. The T2 of the $MnCl_2$ containing phantom was 199 ± 2 ms. The coefficient of variability was < 0.6% for both sequences.

3.6.1.1.2. Spin echo and EPI T2 relaxometry

Results from the 14 infants who had both SE and EPI T2 relaxometry are shown in Figure 3.14. The difference between both methods was assessed using the Bland-Altman method (mean difference of 0.82% with a lower limit of –5.03% (95% CI –8.05% to-2.01%) and an upper limit of 6.67% (95% CI 3.65% to 9.6%). The EPI SE sequence was preferred for 2 reasons: (i) the shorter acquisition time (1.42 min vs. 11 min) resulted in fewer motion and registration artefacts, and (ii) the slice positions for both T2 relaxometry and DWI were equivalent allowing analysis of the same tissue by both methods.

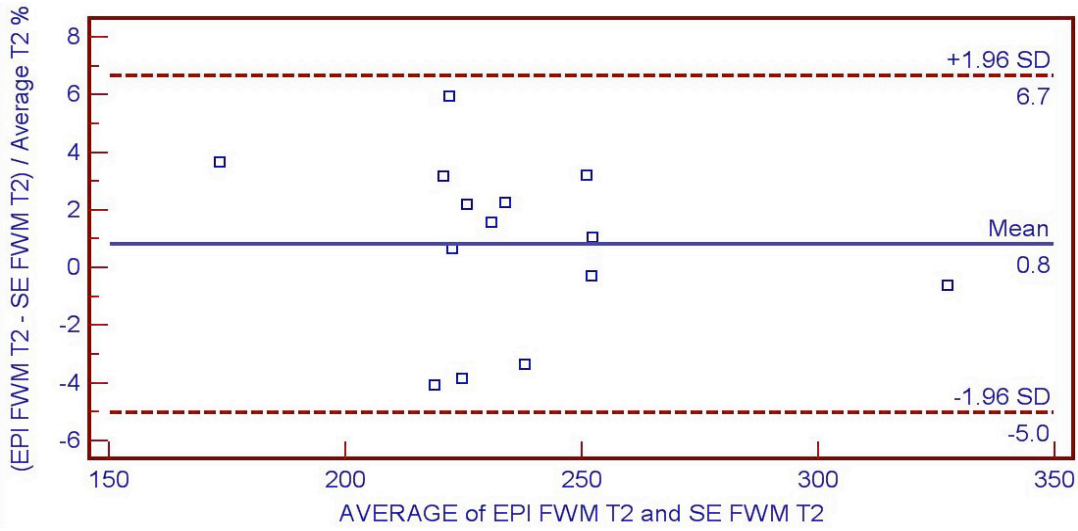


Fig. 3.14. Bland-Altman plot showing the relative percent differences between EPI and SE T2-relaxometry results against their mean in 14 infants who were studied by both methods.

3.6.1.2. Inter-and intra-observer agreement of T2 measurements

The κ statistics for inter-observer and intra-observer agreement for positioning T2 ROIs was high, representing substantial agreement (κ=0.68 and κ=0.77 respectively).

3.6.2. T2 measurements in all preterm infants

3.6.2.1. WM T2 measurements

Six infants had to be excluded from T2 analysis because of movement artefacts. Table 36 shows the WM T2 at the level of the CSO (Fig.3.15a): T2 was measured in the anterior, central and posterior WM. At the level of the basal ganglia, T2 was measured in the periventricular WM anteriorly (pv AWM), in AWM uniform and PWM uniform) (Table 34).

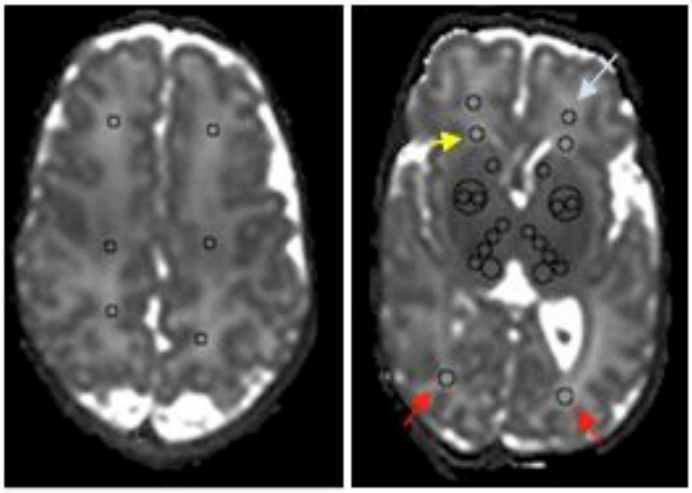


Fig.3.15a and b. T2 map at the level of the CSO with ROIs in the AWM, CWM and PWM; b. T2 map at the level of the basal ganglia/thalami with ROIs in the BG and Thalami and in the periventricular (blue arrow) and uniform (yellow arrow) looking WM anteriorly and posteriorly (red arrow).

3.6.2.1.1. WM T2 measurements at the level of CSO

No significant differences were found between right and left hemispheric T2 in the anterior and central WM at the level of CSO (Table 3.36), hence for further analysis mean T2 of both hemispheres was calculated. Since the right PWM T2 was significantly longer than the left PWM T2, right and left PWM T2 were used in further calculations when all preterm infants were analysed. Fig. 3.16 shows the relation of AWM, CWM and PWM T2. The longest T2 was found in the right PWM. The PWM T2 was significantly longer than CWM or AWM T2 ($p<0.01$). AWM T2 was significantly longer than CWM T2 ($p<0.01$).

ROIs	Mean T2 (ms)	SD	Range (ms)	*p*
AWM right	257.6	33.2	174.3- 340.9	
AWM left	257.6	36.7	183.6- 356.1	*0.99*
CWM right	236.7	42.5	145.7- 368.0	
CWM left	237.1	40.3	169.8- 353.8	*0.53*
PWM right	292.5	56.6	141.7- 456.7	
PWM left	283.7	51.1	167.9- 446.9	*0.02*

Table 3.36. Mean WM T2 (SD, range) at the level of the CSO. *p* is the difference between right and left hemisphere

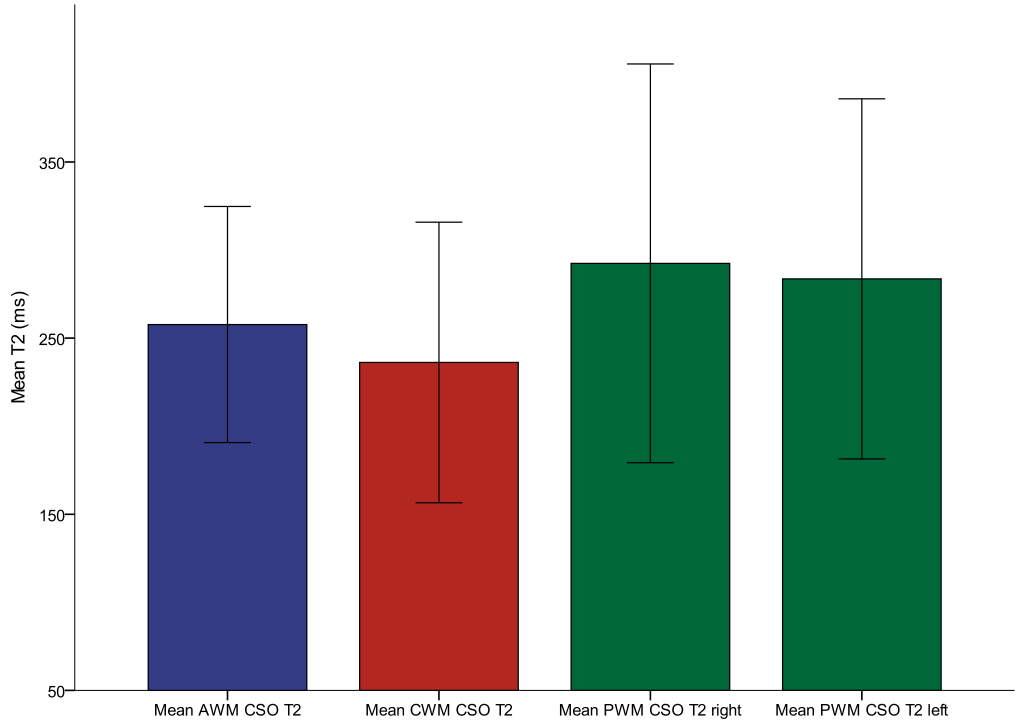


Fig.3.16. Bar chart showing mean T2 in AWM, CWM and right and left PWM. Longest T2 values were found in the right PWM. Error bars ±2SD

3.6.2.1.2. WM T2 measurements at the level of basal ganglia/thalami

No significant differences were found in the WM T2 between both hemispheres (Table 3.37), hence for further analysis mean T2 of both hemispheres were calculated (Table 3.38). The longest mean T2 was found in the uniform AWM. Uniform AWM T2 was significantly longer than periventricular AWM T2 ($p<0.01$) (Fig.3.17). A significant correlation was found between uniform AWM T2 and uniform PWM T2 with uniform AWM being significantly longer than uniform PWM T2 (*p<0.01*). Periventricular AWM T2 was significantly longer than uniform PWM T2 (*p=0.01*).

ROIs	Mean T2 (ms)	SD	Range (ms)	*p*
AWM pv right	275.7	43.5	174.3- 340.9	
AWM pv left	269.1	38.9	183.6- 356.1	*0.59*
AWM uniform right	297.9	52.6	169.5- 434.5	
AWM uniform left	293.2	46.1	169.8- 353.8	*0.18*
PWM uniform right	260.2	38.9	135.5- 371.0	
PWM uniform left	263.2	45.0	134.0- 395.3	*0.40*

Table 3.37. Mean (SD, range) WM T2 at the level of the basal ganglia/thalami. *p* is the difference between right and left hemisphere

ROIs	Mean T2 (ms)	SD
AWM pv	272.4	38.5
AWM uniform	295.5	47.1
PWM uniform	261.7	39.3

Table 3.38. Mean (SD) WM T2 at the level of the basal ganglia/thalami

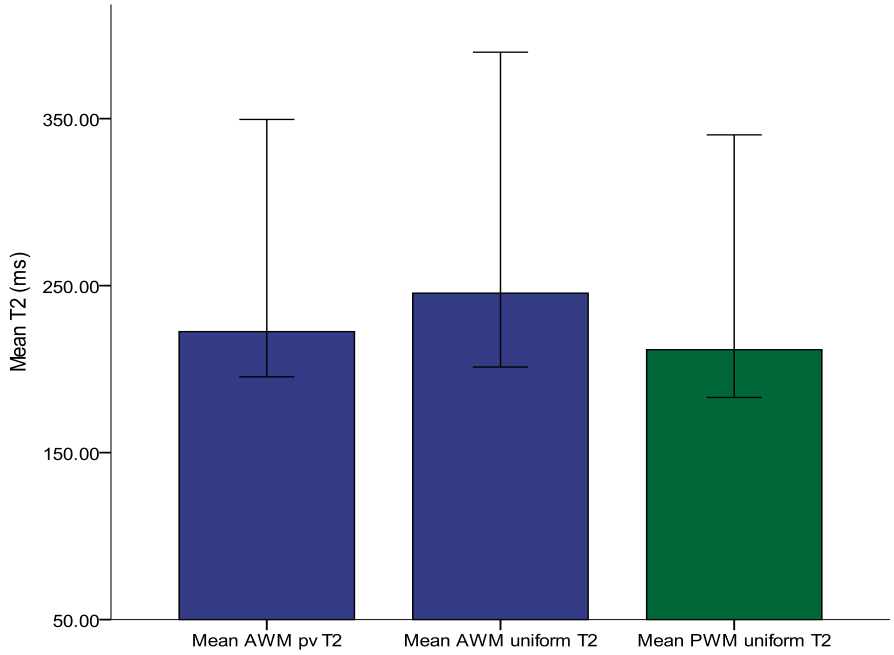


Fig.3.17. Bar chart showing mean T2 in the periventricular AWM, uniform AWM and uniform PWM. Longest T2 was found in uniform AWM. Error bars ±2SD

Of all measured WM T2 was shortest in the central WM at the level of the CSO and longest in the PWM at the level of the CSO and the uniform AWM at the level of the basal ganglia/thalami. Mean T2 values in the CSO PWM (right 292.5ms) and the uniform AWM (295.5ms) were similar.

3.6.2.2. Cerebellar T2 measurements

No significant difference between right and left cerebellar hemisphere T2 (Fig.3.18) was noted; therefore mean T2 of both hemispheres was used in further analysis. Mean cerebellar T2 was 156.5ms (22.9).

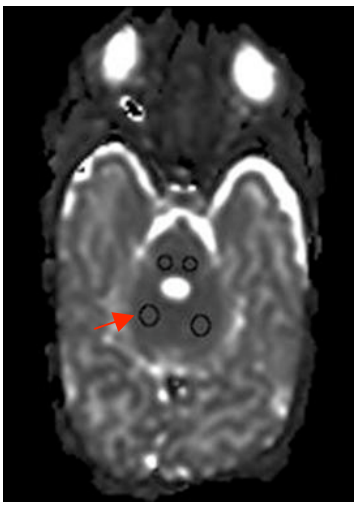


Fig.3.18. T2 map showing ROIs in the cerebellar hemispheres (red arrow)

3.6.2.3. GM T2 measurements

GM T2 was measured in the putamen, globus pallidus, caudate, ventrolateral thalami, thalami and brainstem in both hemispheres (Table 3.39). There were no significant differences between the right and left hemispheric T2 measurements of the globus pallidus, thalami, caudate or brainstem. Left putamen T2 was significantly longer than the right putamen *(p=0.01*) (Table 3.39). All GM T2 values were shorter than any measured WM T2 (*p<0.01*) (Fig.3.19). The longest GM T2 was measured in the caudate: caudate T2 was significantly longer than in any other GM structure (Fig.3.20, table 3.40).

ROIs	Mean T2 (ms)	SD	Range (ms)	*p*
GP right	154.6	10.7	133.6-1 79.8	
GP left	156.2	13.1	123.9- 194.5	0.1
Putamen right	151.3	151.3	131.7- 184.1	
Putamen left	154.5	154.5	133.6- 177.8	0.01
Thalami right	145.9	9.2	116.5- 171.4	
Thalami left	146.7	10.5	124.2- 167.3	0.29
Thalami vl right	155.4	10.6	120.1- 181.7	
Thalami vl left	153.8	11.5	127.3- 176.1	0.15
Caudate right	170.0	13.9	145.0- 221.6	
Caudate left	171.6	14.8	141.4- 225.5	0.40
Brainstem right	133.7	12.9	104.6- 176.3	
Brainstem left	132.9	13.1	103.4- 177.9	0.41

Table 3.39. Mean GM T2 (SD, range) of both hemispheres. *p* is the difference between right and left hemisphere

ROIs	Mean T2 (ms)	SD
GP	155.4	11.1
Thalami	146.3	9.4
Thalami vl	154.3	9.9
Caudate	170.8	13.1
Brainstem	133.3	12.5

Table 3.40. Mean basal ganglia and thalami T2

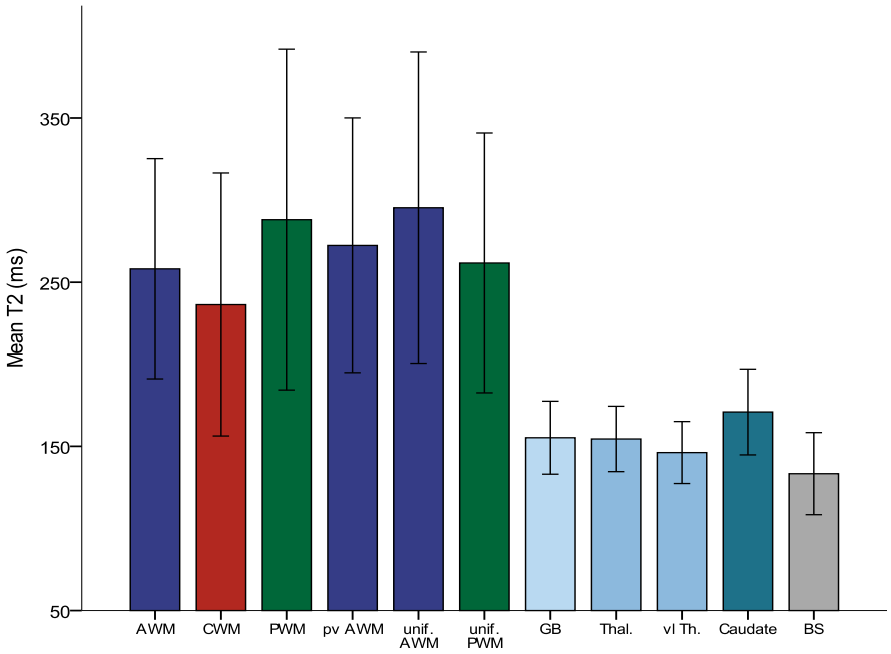


Fig.3.19. Bar chart showing mean T2 in all measured ROIs. Error bars ±2SD

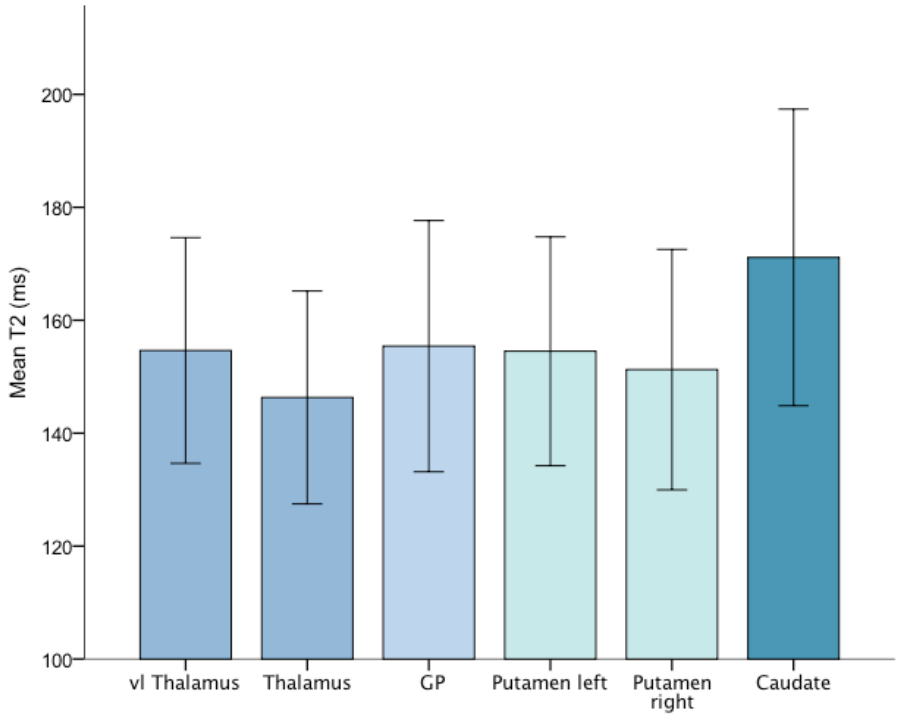


Fig.3.20. Bar chart showing mean T2 in the vl thalami, globus pallidus, putamina and caudate. Longest T2 was found in the caudate. Error bars ±2SD

3.6.3. T2 measurements in preterm infants with cystic lesions

3.6.3.1. WM T2 measurements

18 infants had cystic lesions of which 11 were cPVL and 7 HPI lesions. In 16/18 infants with cystic lesions T2 could be measured. No significant difference was found between right and left WM T2 measurements, therefore mean T2 of both hemispheres was used for further analysis.

ROIs	Mean T2 (ms)	SD	Range (ms)
AWM	259.2	44.2	177.3-345.7
CWM	240.3	48.8	160.1-334.2
PWM	279.4	69.5	154.9-451.8
AWM pv	259.2	47.3	165.4-355.3
AWM uniform	274.9	63.4	177.3-430.5
PWM uniform	257.4	59.7	134.8-339.9

Table 3.41. Mean WM T2 in preterm infants with cystic lesions

Regional variability was found with CSO PWM T2 being significantly longer than CSO AWM T2 (p=0.01) and CSO CWM T2 being significantly longer than AWM T2 (*p=0.03*) (Table 3.41). No regional variability was found in WM T2 at the level of the basal ganglia/thalami. There was no significant difference in any measured WM T2 between infants with cystic WM lesions due to cPVL and preterm infants with cystic WM lesions due to HPI.

3.6.3.2. GM T2 measurements

Table 3.42 shows the GM T2 in infants with cystic lesions. Right and left GM T2 measurements were similar. No differences were found in GM T2 between preterm infants with cystic lesions due to cPVL and preterm infants with cystic lesions due to HPI.

ROIs	Mean T2 (ms)	SD	*p*
GP right	155.1	11.1	
GP left	153.4	15.2	0.4
Putamen right	149.8	10.9	
Putamen left	151.0	8.6	0.6
Thalami right	154.2	13.3	
Thalami left	149.6	12.7	0.2
Thalami vl right	144.2	11.7	
Thalami vl left	143.3	10.4	0.7
Caudate right	168.9	14.3	
Caudate left	169.4	12.5	0.9
Brainstem right	132.4	13.5	
Brainstem left	131.1	13.8	0.6

Table 3.42. Hemispheric GM T2 measurements in preterm infants with cystic lesions. *p* is the difference between right and left hemisphere

3.6.4. T2 measurements in preterm infants without cystic lesions

3.6.4.1. WM T2 measurements

Of the 62 infants WM T2 was measurable in 58 infants. Table 3.42 shows the CSO WM T2 and table 3.43 WM T2 at the level of the basal ganglia/thalami. No significant differences were found between right and left T2 measurements, therefore mean T2 of both hemispheres was used in further analysis. T2 was longest in CSO PWM and uniform AWM at the level of basal ganglia/thalami (Fig.3.21).

ROIs	Mean T2 (ms)	SD	Range (ms)	*p values*
AWM right	256.8	29.6	202.1-330.8	
AWM left	257.5	34.8	214.6-356.1	0.79
CWM right	234.6	40.3	170.1-368.0	
CWM left	235.5	37.6	170.0-353.8	*0.74*
PWM right	294.2	49.9	190.8-402.0	
PWM left	286.7	46.4	208.2-400.8	*0.70*

Table 3.42. Mean CSO WM T2 in preterm infants without cystic lesions. *p* is the difference between right and left hemisphere

ROIs	Mean T2 (ms)	SD	Range (ms)	*p values*
AWM pv right	279.1	40.9	200.4-389.5	
AWM pv left	273.1	35.8	192.9-385.2	*0.14*
AWM uniform right	302.5	47.9	192.7-434.5	
AWM uniform left	299.9	37.9	232.2-396.7	*0.52*
PWM uniform right	260.6	31.7	198.2-343.0	
PWM uniform left	265.1	37.2	170.8-347.3	*0.19*

Table 3.43. Mean WM T2 at the level of the basal ganglia/thalami in preterm infants without cystic lesions. *p* is the difference between right and left hemisphere

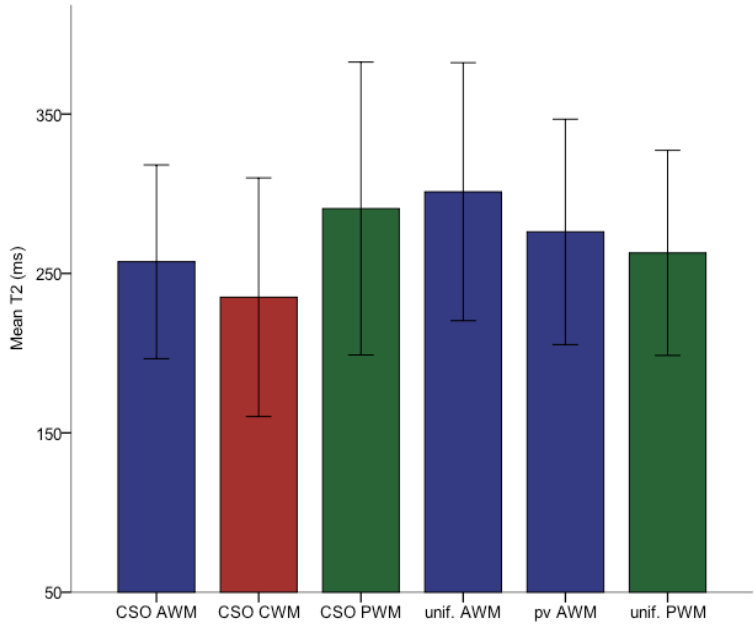


Fig.3.21. Bar chart showing regional variability of mean T2. Error bars ±2SD

A regional difference was seen in CSO WM T2 and in WM T2 at the level of the basal ganglia/thalami. CSO PWM T2 was significantly longer than in the AWM or CWM (*p<0.01*). CSO AWM T2 was significantly longer than CSO CWM T2 (*p<0.01).* At the level of the basal ganglia/thalami uniform AWM T2 was significantly longer than periventricular AWM T2 and uniform PWM T2 (*p<0.01*). Periventricular AWM was significantly longer than uniform PWM T2 (*p<0.01*).

3.6.4.2. GM T2 measurements

Mean interhemispheric T2 values were significantly different in the putamen and globus pallidus. Mean left putaminal T2 was 155.5ms (10.4) compared to 151.6ms (10.6) in the right putamen. Mean left GB T2 was 157.0 ms versus 154.4 ms for the right GB. All other GM and cerebellar T2 values were similar for the right and left hemispheres.

3.6.5. WM/GM T2 differences between preterm infants with and without cystic WM lesions and WM/GM T2 differences between preterm infants with and without DEHSI

There were no significant differences in any of the measured WM T2 values between infants with cystic infants (n=16) and infants without cystic lesions (n=64) apart from the uniform AWM T2. Mean uniform AWM T2 in preterm infants without cystic WM lesions was 301.2ms vs 274.9ms in preterm infants with cystic WM lesions. No significant GM T2 differences were found between preterm infants with and without cystic lesions. All WM T2 values were significantly longer in preterm infants with DEHSI than in those without DEHSI (Table 3.44). In preterm infants with DEHSI mean T2 was longest in the uniform AWM and in CSO PWM (Table 3.44). There were no significant differences in any GM T2 between preterm infants with DEHSI and without DEHSI.

ROIs	PT with DEHSI (n=58)	PT without DEHSI (n=16)	*p value*
AWM	264.9 (32.4)	231.5 (23.7)	*<0.01*
CWM	241.8 (38.8)	215.9 (36.0)	*0.02*
PWM	298.8 (49.1)	249.9 (41.3)	*<0.01*
AWM pv	279.0 (35.9)	248.5 (39.2)	*<0.01*
AWM uniform	303.5 (46.0)	266.6 (40.2)	*<0.01*
PWM uniform	268.2 (33.2)	237.9 (50.6)	*0.04*

Table 3.44. Comparison of WM T2 in preterm infants with and without DEHSI. T2 in ms

Table 3.45 shows WM T2 of preterm infants with DEHSI and preterm infants without DEHSI after exclusion of preterm infants with cystic lesions, and of preterm infants with cystic lesions. WM T2 was longer in preterm infants with DEHSI compared to those without DEHSI. T2 was longest in uniform AWM and CSO PWM. Comparison of WM and GM T2 between preterm infants with DEHSI and preterm infants with cystic lesions was done. WM and GM T2 values were similar in both groups (Table 3.45).

ROIs	PT with DEHSI (n=44)	PT without DEHSI (n=14)	PT with cysts (n=16)	p^1 *value*	p^2 *value*
AWM	263.3	238.4	270.1	*<0.01*	*0.5*
CWM	238.9	223.1	250.9	*0.15*	*0.3*
PWM	300.4	260.3	293.9	*<0.01*	*0.7*

AWM pv	281.3	258.8	271.0	*0.02*	*0.3*
AWM uniform	308.4	278.8	288.4	*<0.01*	*0.2*
PWM uniform	266.5	251.4	273.6	*0.18*	*0.5*

Table 3.45. Comparison of WM T2 in preterm infants with DEHSI, without DEHSI and with cystic lesions. p^1 is difference between WM T2 of preterm infants with and without DEHSI, p^2 is difference between preterm infants with DEHSI and preterm infants with cystic lesions. T2 in ms

There were no GM T2 differences between preterm infants with or without DEHSI. Cerebellar T2 was significantly longer in preterm infants with DEHSI than in preterm infants without DEHSI (160.9ms vs 148.9ms).

3.6.6. Correlation between WM/GM T2 and gestational age/corrected gestational age at MRI scanning in all preterm infants

No correlation was found between gestational age and any GM T2 or WM T2 (Fig.3.22a and b). All GM T2 values correlated significantly with corrected gestational age at MR scanning (Fig.3.23a) Only uniform PWM T2 correlated significantly with corrected gestational age at MR scanning (*p=0.028*, regression coefficient (95%CI) -6.4 (-12.0 to -0.70), no other WM T2 measurement correlated (Fig.23b).

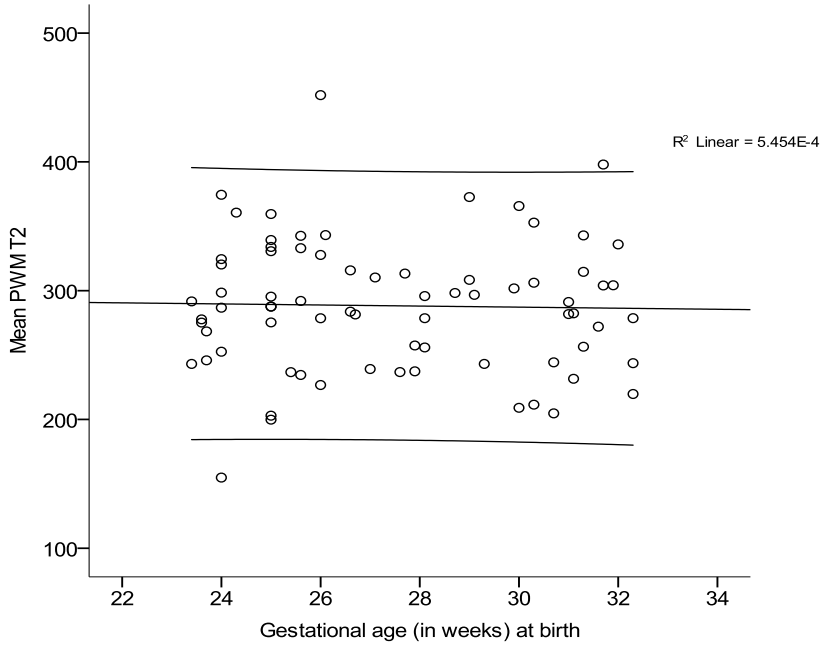


Fig.3.22a. Scatterplot showing the correlation between mean PWM T2 and gestational age at birth

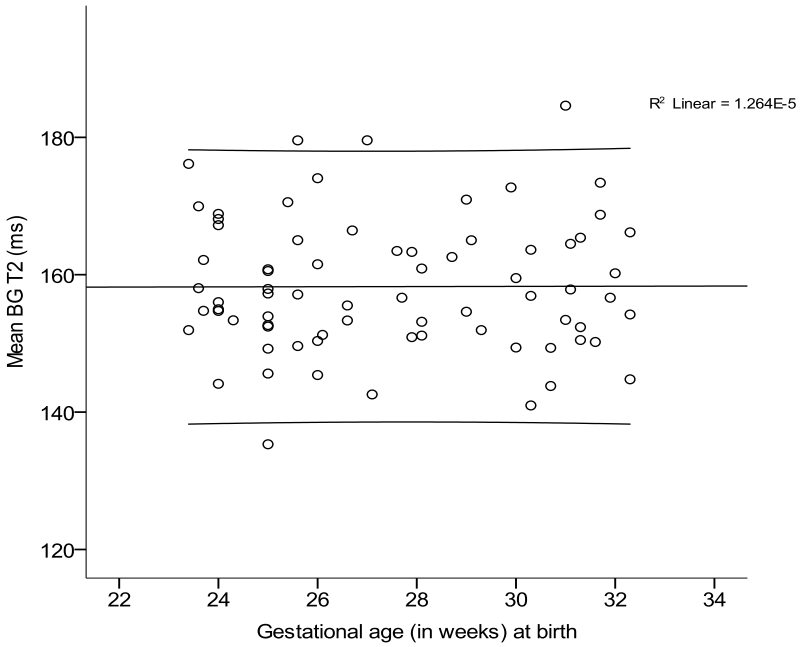


Fig.3.22b Scatterplot showing the correlation mean BG T2 and gestational age at birth. Inner line represents the linear fit, outer lines CI 95%

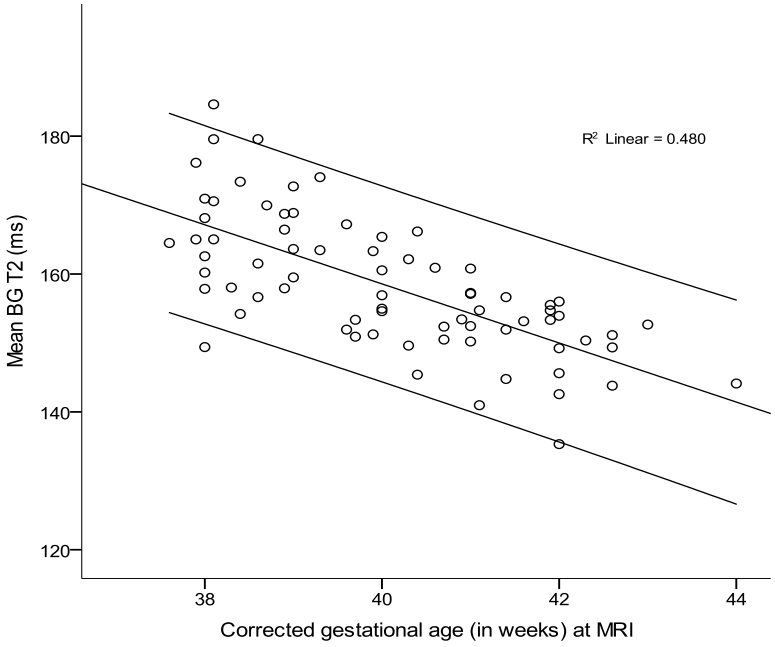


Fig.3.23a. Scatterplot showing a negative linear correlation between mean BG T2 and corrected gestational age at scanning. Inner line represents the linear fit, outer lines CI 95%

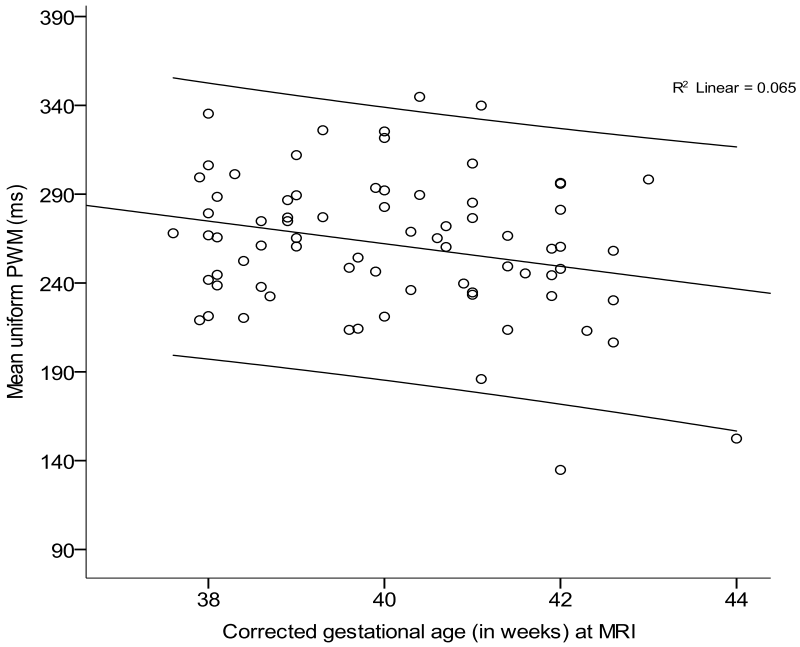


Fig.3.23b. Scatterplot showing the correlation between mean uniform PWM T2 and corrected gestational age at scanning.

The negative correlation between BG T2 and corrected gestational age remained significant when preterm infants with cystic lesions or preterm infants without cystic lesions were analysed separately. The correlation between uniform PWM T2 and corrected gestational age became insignificant when preterm infants with cystic lesions or preterm infants without cystic lesions were analysed separately ((*p=0.07*, regression coefficient (95%CI) -15.1(-31.7 to 1.5)) and (*p=0.30*, regression coefficient (95%CI) -2.9 (-8.6 to 2.7)) respectively).

3.6.7. T2 measurements in control infants

WM and GM T2 of all control infants are shown in table 3.46. All WM T2 were higher than any GM T2 (Table 3.46). No regional T2 variability could be found in measured WM regions. Mean caudate T2 was longest within measured GM regions. No correlation was found between T2 and gestational age at birth. A significant correlation was found between WM/GM T2 and corrected age at MRI, however, as n=5, hence very small, this has to be taken with caution (Fig.3.24a and b).

ROIs	Control infants (n=9)
AWM	222.1 (24.8)
CWM	212.4 (26.1)
PWM	221.4 (22.4)
AWM pv	232.1 (28.9)
AWM uniform	232.1 (32.7)
PWM uniform	229.9 (20.9)
vl thalamus	137.3 (5.5)
GP	145.7 (4.8)

Putamen	141.8 (7.2)
Caudate	161.7 (11.0)
Cerebellum	165.3 (18.6)
Brainstem	140.0 (13.0)

Table 3.46. Mean (SD) T2 in WM and GM ROIs in control infants

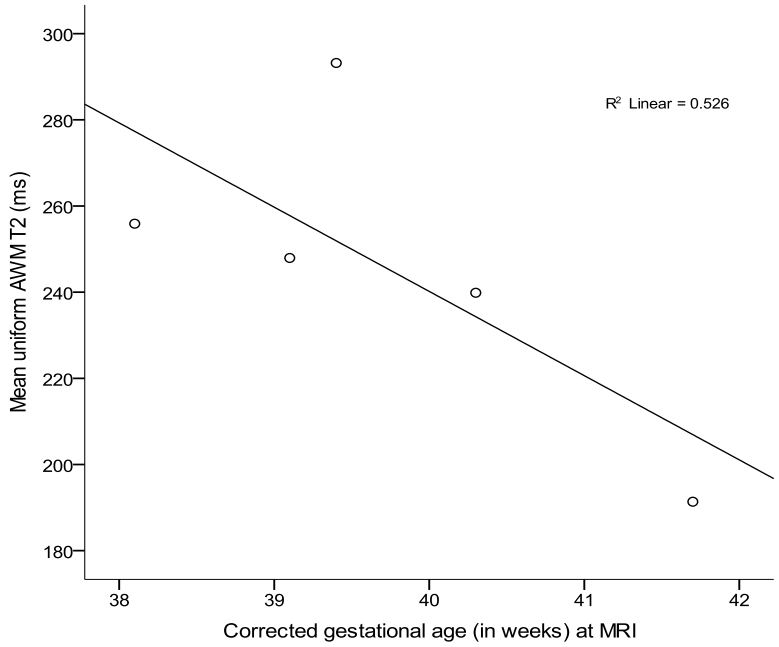


Fig.3.24a. Scatterplot showing linear correlation between mean uniform AWM T2 and corrected gestational age at MRI in control infants

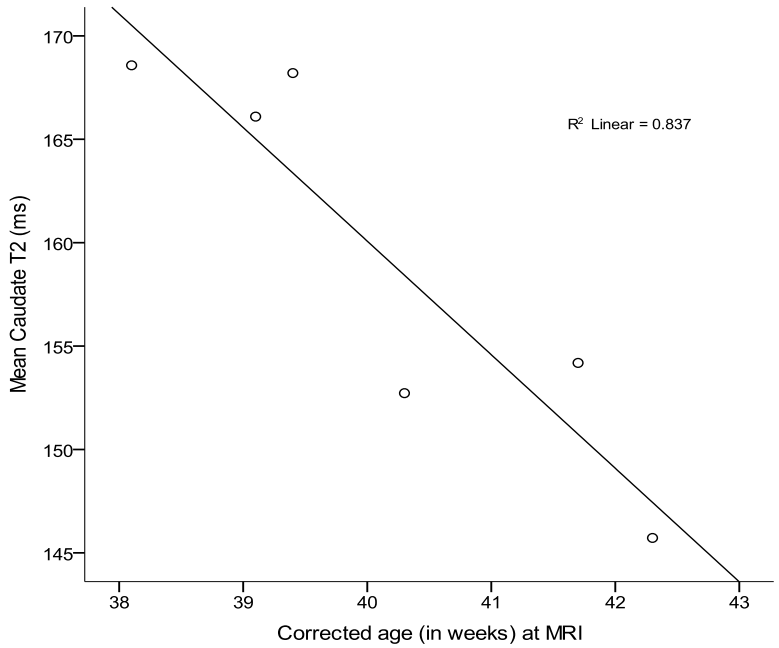


Fig.3.24b. Scatterplot showing a strong correlation between mean caudate T2 and corrected gestational age at MRI in control infants

3.6.7.1. Comparison of T2 measurements between control and preterm infants

Mean WM T2 was significantly longer in preterm infants than in control infants with the exception of mean CSO CWM T2 (Table 3.47). Mean right and left putamen T2, mean GP T2 and mean vl thalamus T2 were significantly longer in preterm infants than in control infants (Table 3.47). Mean brainstem T2 and mean cerebellar T2 were similar in preterm and control infants. Mean caudate T2 was longer in preterm infants than in control infants but the difference did not reach significance.

ROIs	Control infants (n=7)	PT (n=74)	*p value*
AWM	222.1 (24.8)	259.0 (35.6)	*<0.01*
CWM	212.4 (26.1)	236.2 (36.4)	*0.05*
PWM left	223.2 (20.8)	283.6 (51.1)	*<0.01*
PWM right	219.5 (24.8)	292.5 (56.6)	*<0.01*
AWM pv	232.1 (28.9)	272.4 (38.5)	*<0.01*
AWM uniform	232.1 (32.7)	295.5 (47.1)	*<0.01*
PWM uniform	229.9 (20.9)	261.7 (39.3)	*<0.01*
vl thalamus	137.3 (5.5)	146.3 (9.4)	*<0.01*
GP	145.7 (4.8)	155.4 (11.1)	*<0.01*
Putamen left	145.1 (7.7)	154.5 (10.1)	*<0.01*
Putamen right	138.6 (10.8)	151.3 (10.6)	*<0.01*
Caudate	161.7 (11.0)	171.1 (13.3)	*0.06*
Cerebellum	165.3 (18.6)	156.4 (23.0)	*0.23*
Brainstem	140.0 (13.0)	133.3 (12.5)	*0.27*

Table 3.47. Mean WM and GM T2 in control and preterm infants. T2 in ms

No statistically significant difference in mean WM T2 could be found between control infants and preterm infants without DEHSI whereas mean WM T2 values were significantly longer in all measured WM regions in preterm infants with DEHSI compared to control infants (Table 3.48). GM T2 values were similar in preterm infants without DEHSI and in control infants whereas preterm infants with DEHSI had significantly longer GM T2 than control infants.

ROIs	Control infants (n=7)	PT without DEHSI (n=16)	PT with DEHSI (n=58)	p^1 values	p^2 values
AWM	222.1 (24.8)	231.5 (23.7)	264.9 (32.4)	*0.41*	*<0.01*
CWM	212.4 (26.1)	215.9 (36.0)	241.8 (38.8)	*0.63*	*0.02*
PWM	221.4 (22.4)	249.9 (41.3)	298.8 (49.1)	*0.05*	*<0.01*
AWM pv	232.1 (28.9)	248.5 (39.2)	279.0 (35.9)	*0.3*	*<0.01*
AWM uniform	232.1 (32.7)	266.6 (40.2)	303.5 (46.0)	*0.23*	*<0.01*
PWM uniform	229.9 (20.9)	237.9 (50.6)	268.2 (33.2)	*0.56*	*<0.01*

Table 3.48. Differences of T2 in control infants and preterm infants with and without DEHSI. T2 in ms. p^1 is significance between control infants and preterm infants without DEHSI; p^2 is between control infants and preterm infants with DEHSI.

3.6.8. Correlation of T2 measurements with clinical data

Mean AWM T2 correlated with gestational age at birth, chorioamnionitis and postnatal hydrocortisone administration on univariate analysis; on multiple linear regression chorioamnionitis and postnatal hydrocortisone administration remained significantly correlated with mean AWM T2. Mean PWM T2 correlated with gestational age at birth and birth weight. Mean pv AWM T2, mean uniform AWM T2 and mean uniform PWM T2 correlated with chorioamnionitis. Mean GP, mean putamen, mean thalamus and mean vl thalamus correlated with birth weight. Mean vl thalamus and mean thalamus correlated with birth weight.

3.6.9. Correlation of T2 measurements with neurodevelopmental outcome

3.6.9.1. At one year

3.6.9.1.1. In all preterm infants

Correlations between composite cognitive, motor and language scores, receptive and expressive communication scaled scores and gross and fine motor scaled scores for all preterm infants with one year FU were done (n=61). Uniform PWM T2 was negatively correlated with composite motor score (*p=0.04,* regression coefficient (95%CI) -0.09 (-.19 to -0.003)) and with composite cognitive score (p=0.03, regression coefficient (95%) -0.11 (-0.2 to -0.009), even after correction for gestational age and corrected gestational age at MRI. No correlation was found with composite language score or with any scaled score.

3.6.9.1.2. In preterm infants without cystic lesions

WM T2 correlated with cognitive, motor and language outcome at one year corrected gestational age. Figures 3.25 shows a regression plot of uniform PWM T2 and in a. composite cognitive score. The distribution of the residuals looks normal. On univariate analysis uniform PWM T2 correlated significantly with composite cognitive score, composite motor score, with receptive communication scaled score and gross motor scaled score. As outcome score have been shown to correlate with clinical and with imaging data, multiple linear regression was performed to test whether the correlation remained significant (Table 3.48-50). Composite cognitive score, composite motor score, gross motor scaled score and receptive communication scaled score remained significantly correlated with uniform PWM T2 (Table 3.49-51).

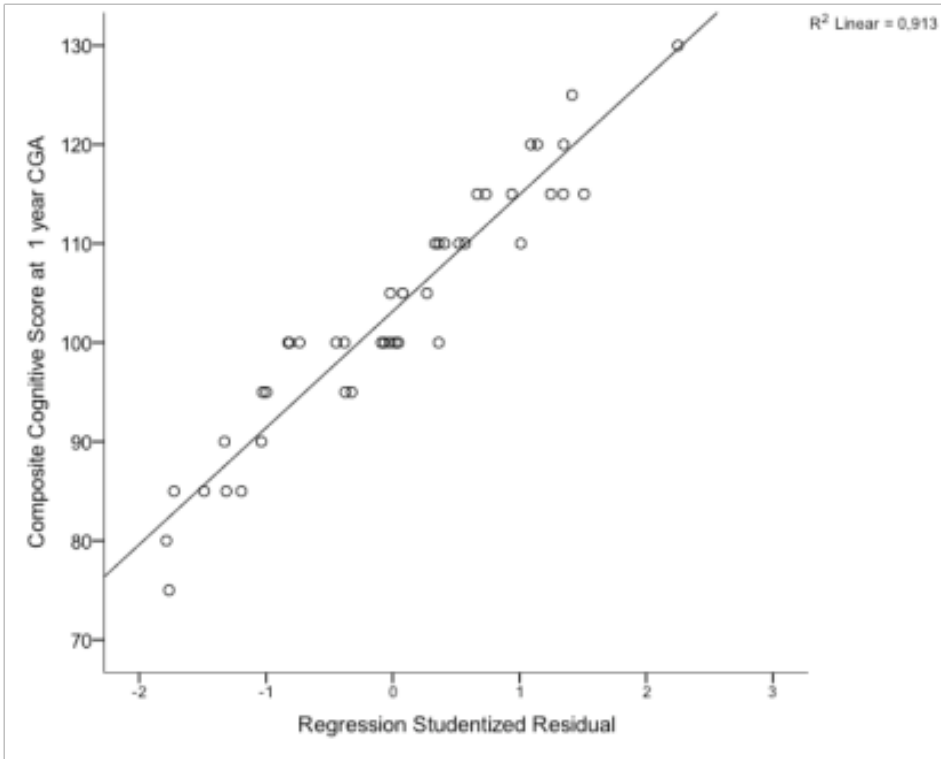


Fig.3.25 Linear regression plots of uniform PWM T2 and composite cognitive scores at one year corrected gestational age showing normal distribution of data

Composite Cognitive **Score**	***p value***	**Regression coefficient (95% CI)**
Uniform PWM T2 (ms)	*0.02*	-0.15 (-0.27 to -0.03)
Cerebellar haemorrhages	*0.12*	11.6 (-2.9 to 26.2)
Birth weight (grams)	*<0.01*	0.02 (0.006 to 0.023)
IVH	*0.7*	-2.1 (-13.1 to 8.8)
DEHSI	*0.5*	2.8 (-6.0 to 11.6)

Table 3.49. Multiple regression of composite cognitive score with cerebellar haemorrhages, birth weight, IVH, DEHSI and uniform PWM T2.

Composite Motor **Score**	***p value***	**Regression coefficient (95% CI)**
Uniform PWM T2 (ms)	*0.04*	-0.14 (-0.3 to -0.001)
Cerebellar haemorrhages	*0.26*	9.1 (-7.0 to 25.3)
Birth weight (grams)	*0.26*	0.005 (-0.005 to 0.015)
IVH	*0.06*	11.6 (-0-4 to 23.7)
DEHSI	*0.93*	0.4 (-9.3 to 10.2)

Table 3.50. Multiple regression of composite motor score with cerebellar haemorrhages, birth weight, IVH, DEHSI and uniform PWM T2.

Composite Language **Score**	***p value***	**Regression coefficient (95% CI)**
Uniform PWM T2 (ms)	*0.08*	-0.12 (-0.26 to 0.018)
Cerebellar haemorrhages	*0.03*	21.0 (2.1 to 39.9)
Birth weight (grams)	*0.19*	0.007 (-0.004 to 0.17)
IVH	*0.38*	-8.5 (-28.3 to 11.3)
DEHSI	*0.51*	3.31 (-6.52 to 12.78)

Table 3.51. Multiple regression of composite language score with cerebellar haemorrhages, birth weight, IVH, DEHSI and uniform PWM T2

However, this correlation seems clinically insignificant (Fig.3.25)

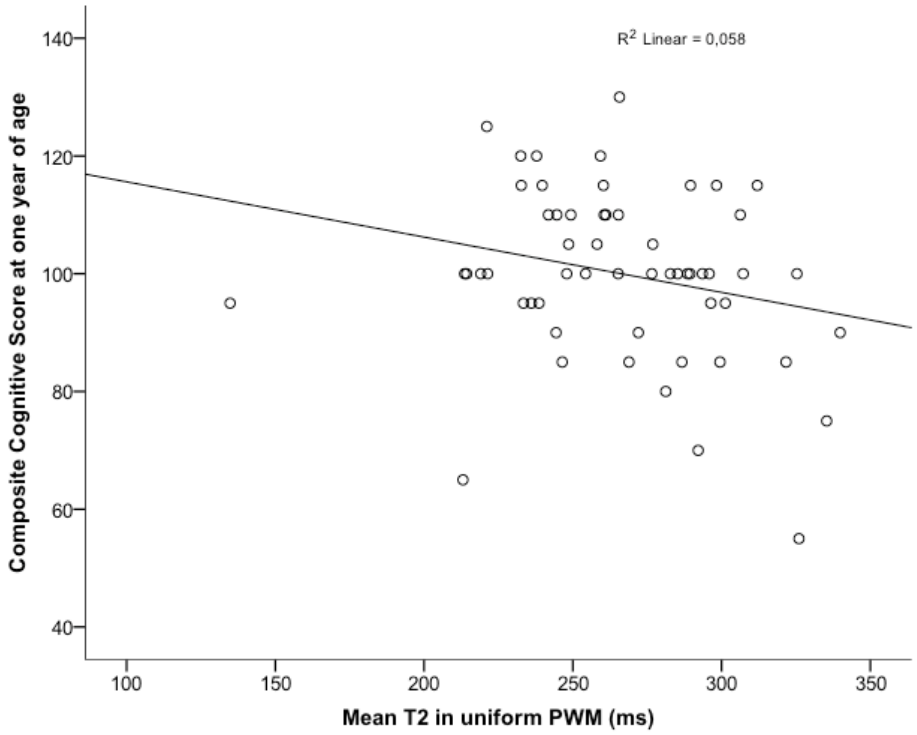


Fig.3.26. Scatterplot showing correlation between uniform PWM T2 and cognitive outcome

3.6.9.2. At two years

3.6.9.2.1. In all preterm infants and with correction for cystic WM lesions

Mean AWM T2, mean PWM T2, pvAWM T2, uniform AWM T2 and uniform PWM T2 correlated significantly with composite cognitive scores, but no correlation was found between mean CWM T2 and composite cognitive scores. These correlations remained significant after adjustment for birth weight, cerebellar haemorrhages, GMH-IVH and for cystic WM lesions. Mean caudate T2 and mean thalamus T2 correlated significantly with composite cognitive scores. After correction for birth weight, cerebellar haemorrhages, GMH-IVH and for cystic WM lesions these correlations became insignificant (*p=0.05* and *p=0.07* respectively). When all T2 measurements, MR variables and BW/GA were put into stepwise linear regression, then mean pv AWM, HPI and IVH remained significant for composite cognitive outcome (Table 3.52).

Composite Cognitive Score	*p value*	Regression coefficient (95% CI)
Mean pv AWM T2 (ms)	*<0.01*	0.19 (0.1 to 0.3)
HPI	*<0.01*	-17.2 (-27.8 to -6.2)
IVH	*<0.01*	-15.8 (-22.7 to -8.3)

Table 3.52. Linear regression between cognitive outcome and WM T2

No significant correlations were found between any WM T2/GM T2 and composite language scores on univariate analysis. There was a trend of correlation between mean uniform AWM T2 and composite language score (*p=0.05)* and between mean caudate T2 and composite language scores (*p=0.06*). On stepwise linear regression (birth weight, MR variables, WM T2) mean caudate T2 remained significantly correlated with language outcome (p<0.01, regression coefficient 0.43 (95%CI 0.09 to 0.7)).

Receptive communication scaled scores correlated significantly with mean caudate T2 (p=0.01), even after adjustment with stepwise linear regression for GA, gender, birthweight, all MR variables and all WM T2. BW remained significant as well (p<0.01).
Expressive communication scaled scores correlated with mean uniform PWM, mean GP T2, mean putamen T2 and mean caudate T2. After adjustment for GA, gender, birthweight, all MR variables and all WM T2, caudate T2 and small CC remained significantly correlated with expressive communication outcome (p<0.01, regression coefficient 0.08 (95%CI 0.03 to 0.1).

Mean AWM T2, mean caudate T2 and mean cerebellar T2 were correlating with composite motor score. On stepwise linear regression (birth weight, all MR variables, WM T2 and basal ganglia T2) mean caudate T2 remained significantly correlated with motor outcome (Table 3.53).

Composite Motor Score	***p value***	**Regression coefficient (95% CI)**
Mean caudate T2	*<0.01*	0.46 (0.2 to 0.7)
Small CC	*<0.01*	-11.2 (-19.3 to -3.1)
Birth weight (grams)	*0.01*	0.02 (0.007 to 0.03)

Table 3.53. Linear regression between motor outcome and WM T2, cerebellar haemorrhages, birth weight, small CC after correction for cystic WM lesions

Significant correlations were found between mean AWM mean putamen T2, mean caudate T2 and mean cerebellar T2 and gross motor scaled scores. On stepwise linear regression (birth weight, all MR variables, WM T2 and basal ganglia T2) mean caudate T2 remained significantly correlated with motor outcome (Table 3.54).

Gross motor scaled score	***p value***	**Regression coefficient (95% CI)**
Mean caudate T2 (ms)	*<0.01*	0.1 (0.05 to 0.1)
Small CC	*0.03*	-1.5 (-2.9 to -0.2)
Birth weight (grams)	*<0.01*	0.03 (0.001 to 0.006)

Table 3.54. Linear regression between motor outcome and T2

Mean pv AWM T2 and mean caudate T2 were significantly correlated with fine motor scaled scores. The correlation between mean pv AWM T2 and fine motor outcome

was significant on stepwise linear regression (adjustment for MR variables, WM and basal ganglia T2, BW/GA) (Table 3.55).

Fine motor scaled score	*p value*	Regression coefficient (95% CI)
pv AWM T2 (ms)	*0.03*	0.02 (0.003 to 0.04)
Small CC	*<0.01*	-2.6 (-4.4 to -0.9)
Birth weight (grams)	*0.03*	0.03 (0.003 to 0.006)

Table 3.55. Linear regression between fine motor outcome and T2

Short Summary:

WM T2 were higher than GM T2 in subgroups. T2 values were significantly higher in preterm infants than in control infants. Preterm infants without DEHSI had similar T2 to control infants whereas preterm infants with DEHSI had significantly longer T2 than those without DEHSI. Regional variability of T2 was found in preterm infants with longest T2 in PWM at the level of CSO and longest T2 in pvAWM at the level of the basal ganglia.

Mean pvAWM correlated with cognitive and fine motor outcome, mean caudate T2 with motor outcome, gross motor outcome and receptive and expressive communication scaled scores at two years of age,

3.7. ADC measurements

3.7.1. ADC measurements in preterm infants

3.7.1.1.1. WM ADC measurements

In 79/80 preterm infants ADC could be measured. There were no differences in ADC between right and left hemispheric measurement (Fig.3.27), hence, mean ADC of both hemispheres was used for further analysis. Regional variability was seen: highest mean ADC values were measured in the PWM. Mean PWM ADC was significantly higher than mean CWM ADC or mean AWM ADC. Mean AWM ADC was significantly higher than mean CWM ADC (Table 3.56).

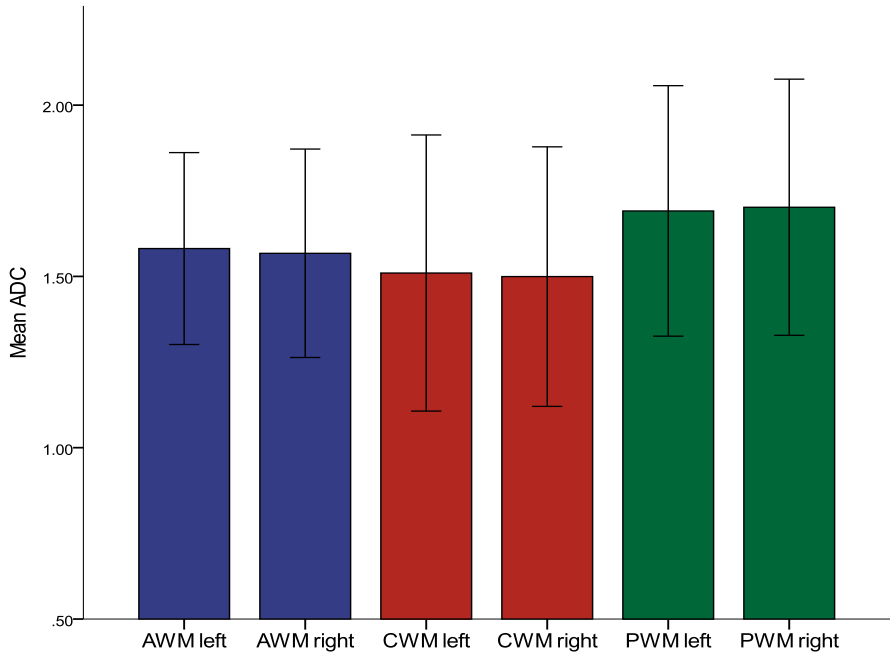


Fig.3.27. Bar chart showing hemispheric CSO WM ADC. ADC in 10^{-3} mm^2/s

ROIs	Mean ADC (10^{-3} mm^2/s)	SD	*p values*
AWM	1.57	0.14	*<0.01*[1]
CWM	1.49	0.19	*<0.01*[2]
PWM	1.69	0.18	*<0.01*[3]

Table 3.56. Mean CSO WM ADC. p^1 shows the significant difference between mean AWM ADC and mean CWM ADC, p^2 shows the difference between mean CWM ADC and mean PWM ADC and p^3 shows the difference between mean AWM ADC and mean PWM ADC.

WM ADC values measured at the level of the basal ganglia/thalami are shown in Table 3.57. As there were no significant differences between right and left hemispheric measurements, mean ADC was calculated. Regional variability was found with uniform AWM being higher than posterior WM ADC.

ROIs	Mean ADC (10^{-3} mm^2/s)	SD	*p values*
AWM pv	1.66	0.14	$<0.01^1$
AWM uniform	1.75	0.15	$<0.01^2$
PWM uniform	1.60	0.14	$<0.01^3$

Table 3.57. Mean WM ADC at the level of the basal ganglia/thalami. p^1 shows the significant difference between mean AWM pv ADC and mean AWM uniform ADC, p^2 shows the difference between mean AWM pv ADC and mean PWM uniform ADC and p^3 shows the difference between mean AWM uniform ADC and mean PWM uniform ADC

3.7.1.2. GM ADC measurements

Mean GM ADC was generally lower than any WM ADC (Fig.3.28). Mean GM ADC values are shown in Table 3.57. Significant ADC differences between right and left were found in the GB and putamen (Table 3.58). Highest GM ADC was found in the caudate.

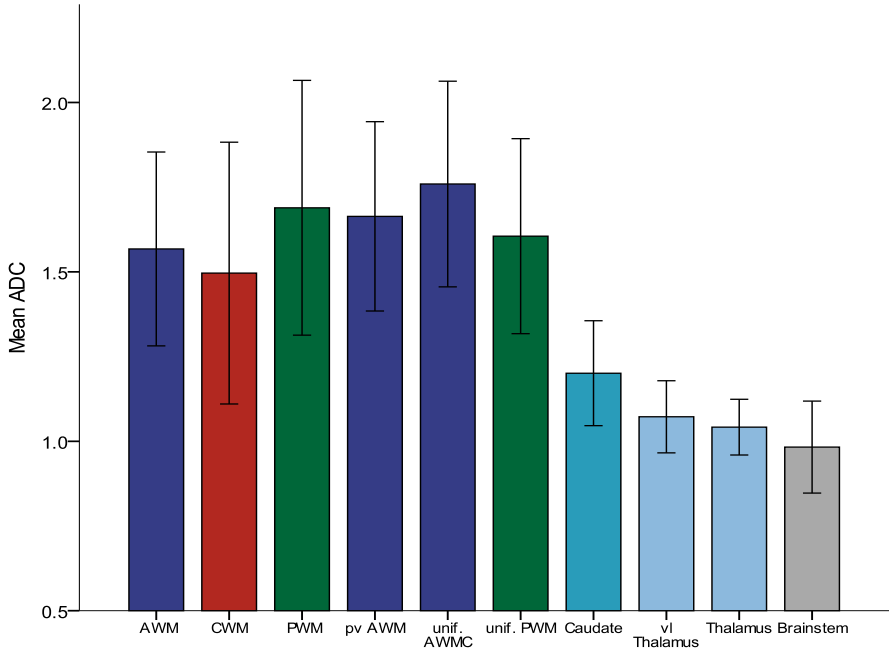


Fig.3.28. Bar chart showing mean ADC in WM and GM regions. ADC in 10^{-3} mm^2/s

ROIs	Mean ADC (10^{-3} mm^2/s)	SD	*p values*
GP right	1.13	0.05	
GP left	1.14	0.05	0.01
Putamen right	1.13	0.06	
Putamen left	1.14	0.06	<0.01
Thalami right	1.07	0.06	
Thalami left	1.06	0.05	0.2
Thalami vl right	1.03	0.04	
Thalami vl left	1.04	0.04	0.06
Caudate right	1.19	0.07	
Caudate left	1.20	0.08	0.4
Brainstem right	0.98	0.06	
Brainstem left	0.98	0.07	0.8

Table 3.58. Mean GM ADC of both hemispheres. *p* is the difference between right and left hemisphere

3.7.1.3. WM/GM ADC differences between preterm infants with and without cystic WM lesions and WM/GM ADC differences between preterm infants with and without DEHSI

Table 3.59 shows WM ADC in preterm infant subgroups: preterm infants with and without cystic lesions and preterm infants with and without DEHSI.

ROIs	PT without cysts (n=61)	PT with cysts (n=18)	PT with DEHSI (n=62)	PT without DEHSI (n=17)
AWM	1.57 (0.13)	1.56 (0.17)	1.59 (0.13)	1.47 (0.13)
CWM	1.49 (0.18)	1.49 (0.23)	1.51 (0.19)	1.43 (0.19)
PWM	1.69 (0.17)	1.65 (0.23)	1.72 (0.17)	1.56 (0.2)
AWM pv	1.67 (0.12)	1.62 (0.18)	1.68 (0.13)	1.60 (0.14)
AWM uniform	1.78 (0.12)	1.68 (0.21)	1.78 (0.13)	1.65 (0.16)
PWM uniform	1.60 (0.11)	1.59 (0.21)	1.63 (0.12)	1.49 (0.16)

Table 3.59. Mean (SD) WM ADC in preterm infant subgroups. ADC in 10^{-3} mm^2/s

62/79 preterm infants with measurable ADC had DEHSI and 18/79 had cystic lesions. 16/18 preterm infants with cystic lesions also had DEHSI. After exclusion of the 18 preterm infants with cystic lesions, comparison of ADC was done between preterm infants with DEHSI (n=46) and preterm infants without DEHSI (n=16) (Table 3.60). All mean WM ADC values were significantly higher in preterm infants with DEHSI than in preterm infants without DEHSI with the exception of mean CWM ADC and mean periventricular AWM ADC. Regional variability was found with mean ADC being highest in the posterior CSO WM and at the basal ganglia/thalami level uniform mean AWM ADC was the highest.

ROIs	PT with DEHSI (n=46)	PT without DEHSI (n=15)	*p value*
AWM	1.58 (0.13)	1.50 (0.10)	*0.02*
CWM	1.50 (0.18)	1.47 (0.18)	*0.63*
PWM	1.72 (0.17)	1.61 (0.14)	*0.01*
AWM pv	1.68 (0.13)	1.64 (0.09)	*0.16*
AWM uniform	1.80 (0.11)	1.70 (0.09)	*<0.01*
PWM uniform	1.63 (0.11)	1.53 (0.10)	*<0.01*

Table 3.60. WM ADC comparison between preterm infants with and without DEHSI after exclusion of 18 preterm infants with cystic lesions. ADC in 10^{-3} mm^2/s

When only preterm infants with cystic lesions were analysed, WM ADC was significantly longer in all measured WM ROIs in preterm infants with DEHSI (n=16) than in preterm infants without DEHSI (n=2); however there were only two infants with cystic lesions and no DEHSI.

Table 3.61 shows mean GM ADC in the different preterm subgroups. Mean ADC remained highest in caudate all subgroups.

ROIs	PT without cysts (n=61)	PT with cysts (n=18)	PT with DEHSI (n=62)	PT without DEHSI (n=17)
GP right	1.13 (0.06)	1.12 (0.03)	1.13 (0.05)	1.11 (0.06)
GP left	1.14 (0.05)	1.13 (0.04)	1.14 (0.05)	1.14 (0.05)
Putamen right	1.13 (0.06)	1.12 (0.05)	1.13 (0.05)	1.12 (0.07)
Putamen left	1.15 (0.06)	1.13 (0.04)	1.14 (0.05)	1.14 (0.07)
Caudate	1.20 (0.08)	1.18 (0.05)	1.20 (0.07)	1.20 (0.09)
Thalamus vl	1.07 (0.05)	1.05 (0.05)	1.07 (0.05)	1.06 (0.04)
Thalami	1.04 (0.04)	1.02 (0.03)	1.05 (0.04)	1.02 (0.04)
Brainstem	0.98 (0.07)	0.96 (0.05)	0.98 (0.07)	0.98 (0.06)

Table 3.61. Mean (SD) GM ADC in preterm infant subgroups. ADC in 10^{-3} mm^2/s

There were no significant differences in mean GM ADC between preterm infants with and without DEHSI even after exclusion of preterm infants with cystic lesions.

3.7.2. Correlation between WM/GM ADC and gestational age/corrected gestational age at MR scanning in all preterm infants

None of the WM ADC values correlated with gestational age at birth (Fig.3.29a). Left and right thalamic showed some correlation with gestational age at birth (*p=0.028*, regression coefficient (95%CI) -0.004 (-0.007 to 0.0) (Fig.3.29b). All GM ADC values correlated with corrected gestational age at MR scanning (Fig. 3.30a). Only uniform PWM ADC correlated minimally with corrected gestational age at MR scanning (*p=0.035*, regression coefficient (95%CI) -0.021 (-0.04 to -0.002) (Fig.3.30b), no other WM T2 measurement correlated.

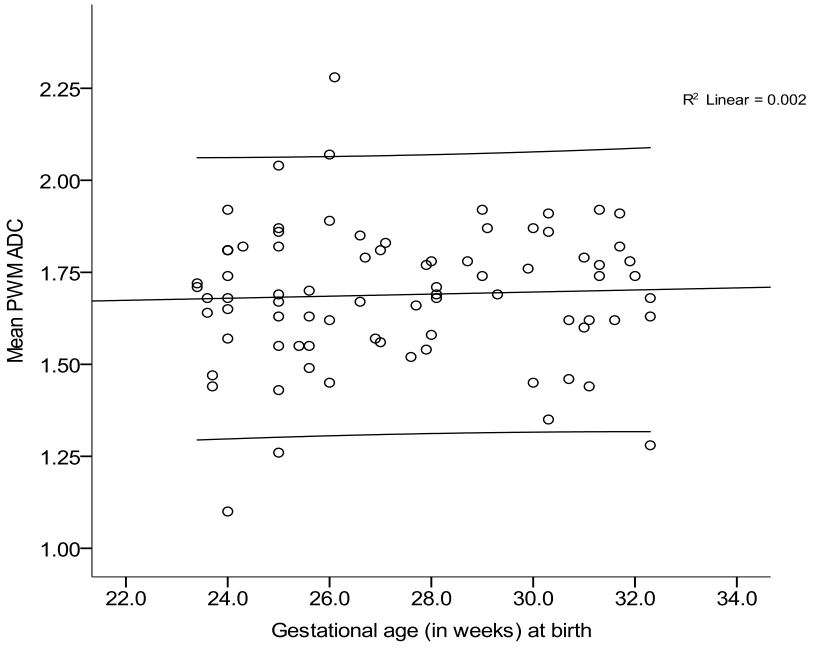


Fig.3.29a. Scatterplot showing no linear correlation between mean PWM ADC and gestational age at birth. Inner line represents the linear fit, outer lines CI 95%. ADC in 10^{-3} mm^2/s

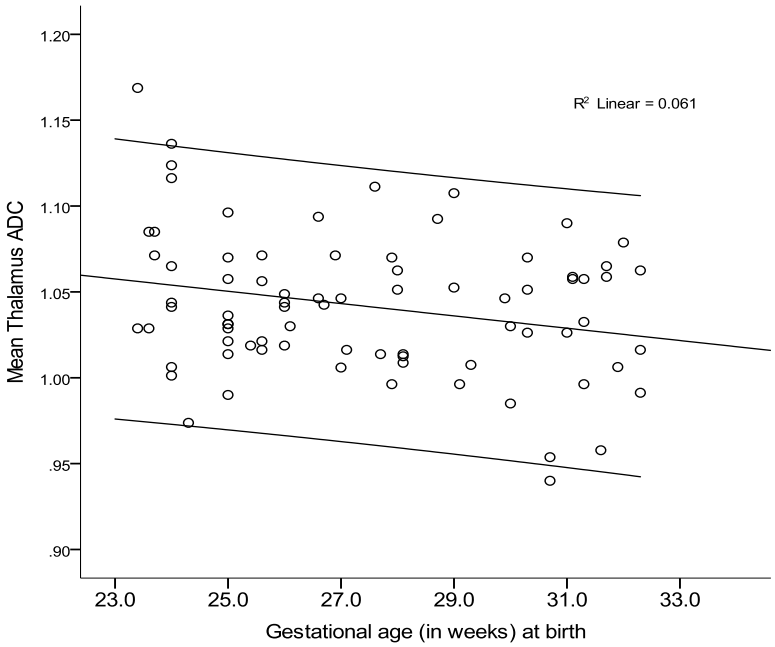


Fig.3.29b. Scatterplot showing weak correlation between mean thalamic ADC and gestational age at birth. Inner line represents the linear fit, outer lines CI 95%. ADC in 10^{-3} mm^2/s

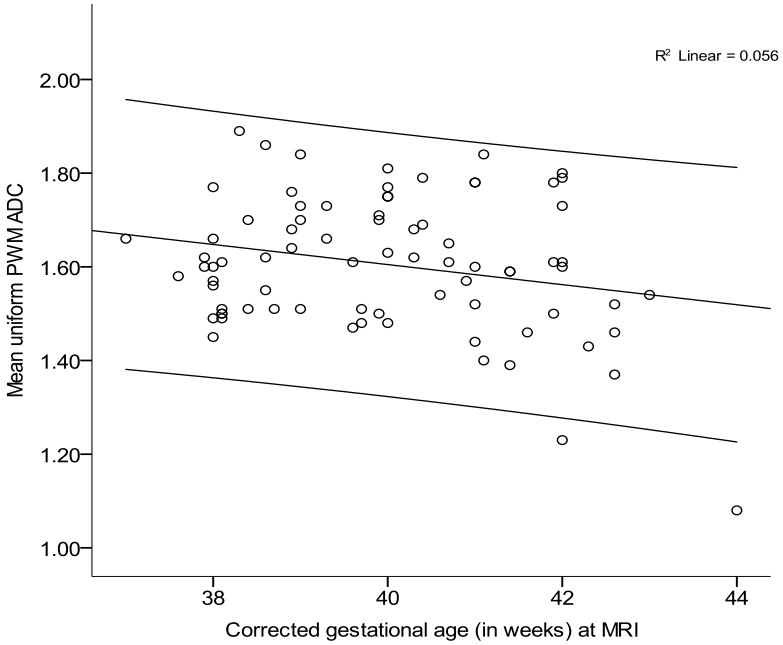


Fig.3.30a. Scatterplot showing minimal linear correlation between mean uniform PWM ADC and corrected gestational age at MR. Inner line represents the linear fit, outer lines CI 95%. ADC in 10^{-3} mm^2/s

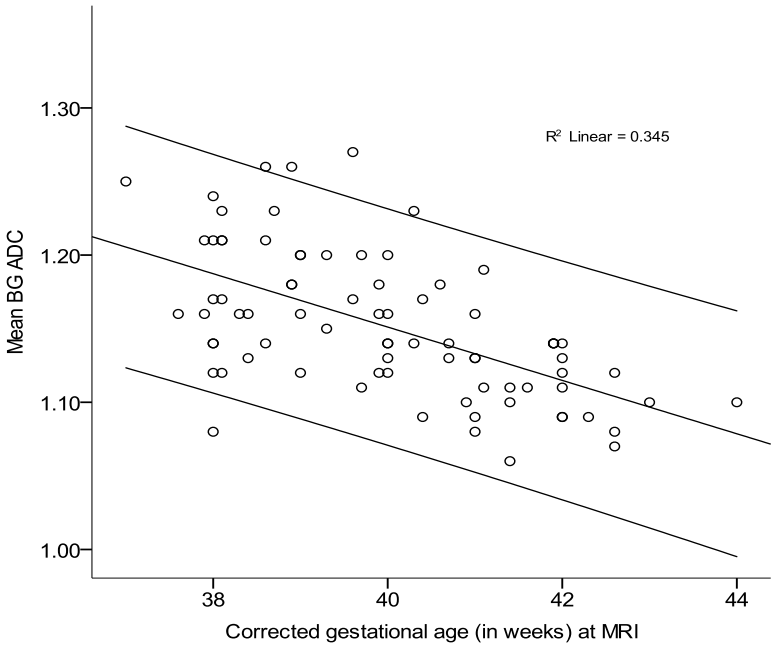


Fig.3.30b. Scatterplot showing moderate correlation between mean BG ADC and corrected gestational age at MRI. Inner line represents the linear fit, outer lines CI 95%. ADC in 10^{-3} mm^2/s

3.7.3. ADC measurements in control infants

In nine control infants WM and GM ADC could be measured, these are shown in table 3.61. All measured mean WM ADC values were higher than any mean GM ADC (Table 3.62). No correlation was found between WM ADC and gestational age at birth or corrected gestational age at MRI. No regional variability between CSO WM regions could be found. At the level of the basal ganglia/thalami, mean uniform AWM ADC was higher than mean pv AWM ADC or CSO PWM ADC. Mean caudate ADC was highest within any measured GM ADC.

ROIs	ADC of control infants (n=9)
AWM	1.46 (0.06)
CWM	1.45 (0.14)
PWM	1.50 (0.09)
AWM pv	1.53 (0.12)
AWM uniform	1.60 (0.10)
PWM uniform	1.49 (0.14)
vl thalamus	1.03 (0.04)
GP	1.12 (0.04)
Putamen	1.12 (0.04)
Caudate	1.18 (0.03)
Cerebellum	1.14 (0.12)
Brainstem	1.04 (0.09)

Table 3.62. Mean (SD) WM and GM ADC measurements. ADC in 10^{-3} mm^2/s

3.7.4. Comparison of ADC measurements between control and preterm infants

Mean WM ADC was significantly higher in preterm infants than in control infants with the exception of mean CSO CWM (Table 3.63). Mean putamen ADC and mean brainstem T2 were significantly higher in preterm infants than in control infants (Table 3.62).

ROIs	Control infants (n=9)	Preterm infants (n=79)	*p value*
AWM	1.46 (0.06)	1.56 (0.14)	*0.03*
CWM	1.45 (0.14)	1.50 (0.19)	*0.46*
PWM	1.50 (0.09)	1.68 (0.18)	*<0.01*
AWM pv	1.53 (0.12)	1.66 (0.14)	*<0.01*
AWM uniform	1.60 (0.10)	1.76 (0.15)	*<0.01*
PWM uniform	1.49 (0.14)	1.61 (0.14)	*0.02*
vl thalamus	1.03 (0.04)	1.04 (0.04)	*0.32*
GP	1.12 (0.04)	1.13 (0.05)	*0.32*
Putamen	1.12 (0.04)	1.14 (0.01)	*<0.01*
Caudate	1.18 (0.03)	1.20 (0.07)	*0.65*
Cerebellum	1.14 (0.12)	1.12 (0.01)	*0.56*
Brainstem	1.04 (0.09)	0.98 (0.07)	*0.02*

Table 3.63. Mean (SD) WM and GM ADC in control and preterm infants. ADC in 10^{-3} mm^2/s

When preterm infants were grouped into preterm infants with DEHSI or without DEHSI, mean ADC in preterm infants with DEHSI was significantly higher in all measured WM regions apart from CSO CWM (Table 3.64). Mean putamen ADC was higher in preterm infants with DEHSI than in control infants (*p<0.01*). Mean brainstem ADC was higher in preterm infants than in control infants (1.15 vs. 1.13) but the difference did not reach significance (*p=0.08*).

No statistically significant difference in mean WM ADC could be found between control infant and preterm infants without DEHSI. Only mean putamen ADC was significantly longer in preterm infants without DEHSI than in control infants, all other GM ADC values were similar between control infants and preterm infants without DEHSI.

ROIs	Control infants (n=9)	PT without DEHSI (n=17)	PT with DEHSI (n=62)	p^1 values	p^2 values
AWM	1.46 (0.06)	1.47 (0.13)	1.59 (0.13)	*0.71*	*<0.01*
CWM	1.45 (0.14)	1.43 (0.19)	1.51 (0.19)	*0.98*	*0.25*
PWM	1.50 (0.09)	1.56 (0.2)	1.72 (0.17)	*0.35*	*<0.01*
AWM pv	1.53 (0.12)	1.60 (0.14)	1.68 (0.13)	*0.19*	*<0.01*
AWM uniform	1.60 (0.10)	1.65 (0.16)	1.78 (0.13)	*0.40*	*<0.01*
PWM uniform	1.49 (0.14)	1.49 (0.16)	1.63 (0.12)	*0.88*	*0.02*

Tabl 3.64. Differences of ADC in control infants and preterm infants with and without DEHSI. ADC in 10^{-3} mm^2/s. p^1 is significance between control infants and preterm infants without DEHSI; p^2 is between control infants and PT with DEHSI.

3.7.5. Correlation between ADC measurements and clinical data

Mean CSO AWM ADC correlated significantly with birth weight (p=0.05, r=0.21) and chorioamnionitis (p=0.01, r=-0.28). On multiple linear regression chorioamnionitis correlated significantly with mean AWM ADC (*p=0.03*, regression coefficient (95% CI) 0.13 (0.03 to 0.23)). Mean uniform AWM ADC correlated with gestational age at birth (p=0.06, r=0.2), birth weight (p=0.02, r=0.25) and chorioamnionitis (p=0.02, r=-0.27). Mean GP correlated significantly with oxygen requirement at 28 days (p=0.03, r=0.25) and at 36 weeks corrected gestational age (p<0.01, r=0.35).

3.7.6. Correlation between ADC measurements and neurodevelopmental outcome

3.7.6.1. At one year

3.7.6.1.1. In all preterm infants

When WM T2 values of all preterm infants were correlated with cognitive, motor and language scores, negative correlation was found between mean uniform AWM ADC and composite cognitive score, even when corrected for gestational age and corrected gestational age at MRI (*p=0.02*, regression coefficient (95% CI) -28.4 (-56.0 to -0.8)). A further negative correlation was found between mean AWM ADC and expressive communication scaled score, even after adjustment for gestational age and corrected gestational age at MRI (*p=0.01*, regression coefficient (95% CI) -6.4 (-11.5 to -1.3).

3.7.6.1.2. In preterm infants without cystic lesions

After exclusion of infants with cystic WM lesions, correlation was performed between WM ADC and outcome measures. Mean AWM ADC correlated significantly with composite language score (*p=0.04*) and expressive communication scaled score (*p<0.01*), but not with receptive communication scaled score. Mean AWM ADC correlated with composite motor score (*p=0.04*), fine motor scaled score (*p=0.03*) but not with gross motor scaled score. Mean CWM ADC did not correlate with any outcome measures. Mean PWM ADC correlated with expressive communication scaled score, even after correction for gestational age at birth and corrected gestational age at MRI (*p=0.04*). Mean uniform PWM ADC did correlate significantly with composite cognitive score (*p<0.01*).

3.7.6.2. At two years

3.7.6.2.1. Univariate linear regression

Mean uniform AWM ADC correlated with composite cognitive scores (*p=0.04*). Mean caudate ADC correlated with composite language scores (*p<0.01*). No correlations were found between receptive communication scaled scores and any measured ADC. Expressive communication scaled scores correlated with mean caudate ADC, mean putamen ADC and mean cerebellar ADC.

Composite motor scores correlated with mean uniform AWM ADC, mean PWM ADC and mean cerebellar ADC. There was a trend between mean caudate ADC and composite motor scores. Gross motor scaled scores were associated with mean uniform AWM ADC, mean CWM ADC, mean caudate ADC, mean putamen ADC and mean cerebellar ADC. No correlations were found between fine motor scaled scores and any measured ADC.

3.7.6.2.2. Stepwise linear regression

When stepwise linear regression was performed, with adjustment for all MR variables, all WM and basal ganglia ADC values, and BW/GA, no ADC measurement correlated with composite cognitive or motor outcome at 2 years. Mean caudate ADC correlated significantly with composite language outcome (Table 3.65).

Composite Language Score	***p value***	**Regression coefficient (95% CI)**
Mean caudate ADC	*0.03*	115.0 (14.3 to 215.8)

Table 3.65. Stepwise linear regression for WM ADC and language outcome

When stepwise linear regression was performed, with adjustment for all MR variables, all WM and basal ganglia ADC values, mean pv AWM ADC correlated significantly with receptive language outcome (Table 3.66).

Receptive communication scaled score	***p value***	**Regression coefficient (95% CI)**
Mean pv AWM ADC	*0.03*	-8.2 (-15.9 to -0.9)
Birth weight	*0.01*	0.004 (0.001 to 0.007)

Table 3.66. Stepwise linear regression for WM ADC and receptive language outcome

Stepwise linear regression showed that mean caudate ADC correlated significantly with expressive language outcome (p=0.02, regression coefficient 18.4 (95%CI 2.8 to 33.9)). No ADC measurement after adjustment for MR variables, WM ADC and BW/GA correlated with fine or gross motor outcome at two years.

Short Summary

ADC values were higher in preterm infants compared to control infants. Preterm infants with DEHSI had significantly higher ADC than those without DEHSI similar to T2 results, however T2 values showed more regional variability than ADC values

Caudate ADC correlated with language outcome, expressive communication outcome and pv AWm ADC with receptive communication outcome.

3.8. FA and eigenvalue measurements

3.8.1. WM FA, EV1, RD and RA measurements in all preterm infants

FA, EV1, RD and RA were measured in the CSO WM, PLIC and in the genu and splenium of the corpus callosum. In 62 infants FA, EV1, RD and RA could be measured. No significant difference was found between right and left AWM, CWM and PWM, hence mean AWM, CWM and PWM measurements of both hemispheres were calculated and used in further analysis. The single significant difference between right and left hemispheric measurements was found in PLIC EV1: right EV1 was larger than the left PLIC (1.03 vs 1.06, $p<0.01$).

ROIs	FA	EV1	*RD*	*RA*
AWM	0.25 (0.05)	1.05 (0.16)	*0.78 (0.08)*	*0.22 (0.0)*
CWM	0.31 (0.06)	1.05 (0.16)	*0.69 (0.10)*	*0.27 (0.07)*
PWM	0.28 (0.06)	1.08 (0.15)	*0.75 (0.09)*	*0.23 (0.06)*
CC genu	0.42 (0.10)	1.40 (0.25)	*0.73 (0.18)*	*0.38 (0.12)*
CC splenium	0.59 (0.19)	1.40 (0.24)	*0.54 (0.33)*	*0.60 (0.24)*
PLIC right	0.52 (0.08)	1.06 (0.13)	*0.46 (0.10)*	*0.48 (0.13)*
PLIC left	0.53 (0.07)	1.04 (0.13)	*0.43 (0.09)*	*0.48 (0.07)*

Table 3.67. Mean FA, EV1, RD and RA values in CSO, corpus callosum (CC) and PLIC

The highest FA of all measured ROIs was found in the splenium of the CC, followed by PLIC FA (Table 3.67, Fig.3.31a). The lowest FA was found in the CSO AWM.
Regional FA variability was found within CSO WM regions and within the corpus callosum regions (Fig.3.31a). CSO CWM FA was significantly higher than AWM FA and PWM FA. PWM FA was significantly higher than AWM FA. FA was significantly higher in the splenium of the CC than in the genu of the CC. No difference was found between right and left PLIC FA.

EV1 was similar in the genu and the splenium of the CC (Fig.3.31b). Regional EV1 variability within CSO WM regions consists of significantly higher EV1 in CSO PWM than in CSO CWM or AWM ($p<0.01$ and $p<0.01$ respectively). CSO AWM EV1 was similar to CSO CWM EV1 ($p=0.63$). Right PLIC EV1 was significantly higher than left PLIC EV1 ($p<0.01$). EV1 of splenium and genu of the CC were similar.

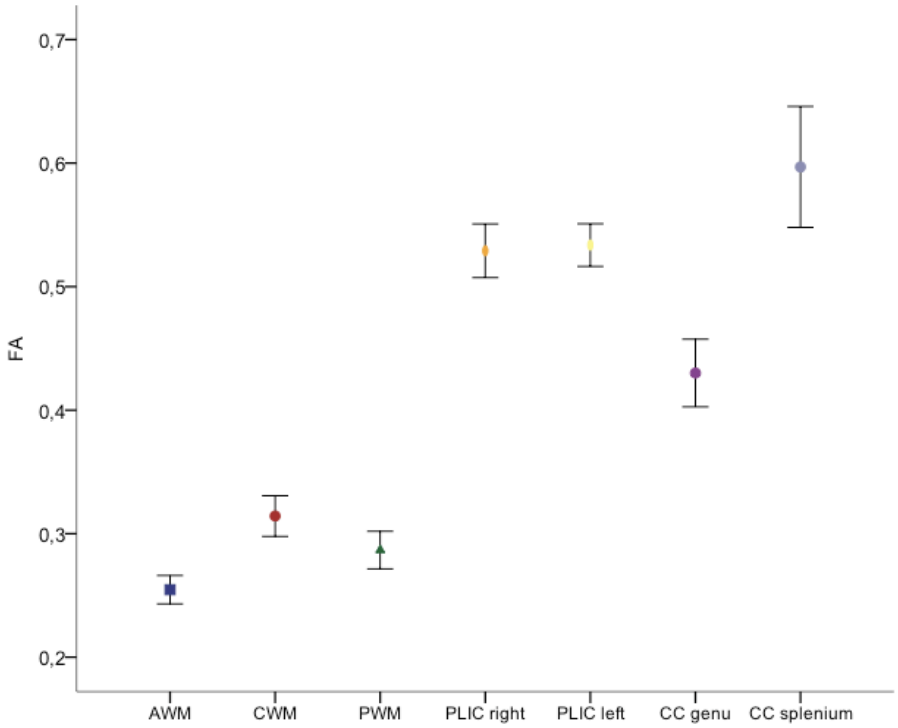


Fig.3.31a. Mean and standard deviations (error bars) of FA in AWM, CWM, PWM, PLIC bilaterally and corpus callosum (CC)

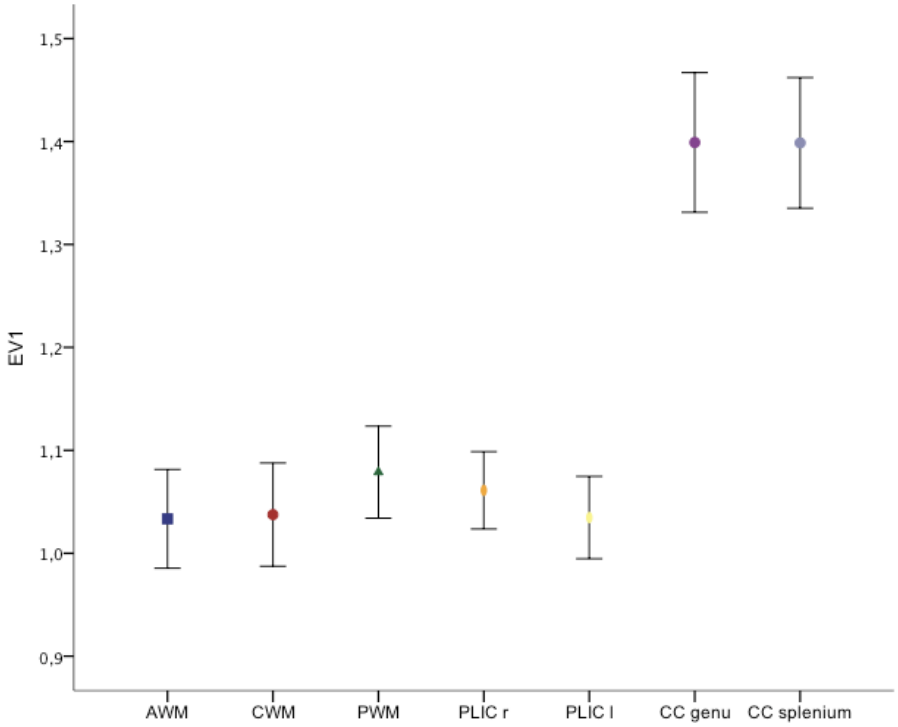


Fig.3.31b. Mean and standard deviations (error bars) of EV1 in AWM, CWM, PWM, PLIC bilaterally and corpus callosum (CC)

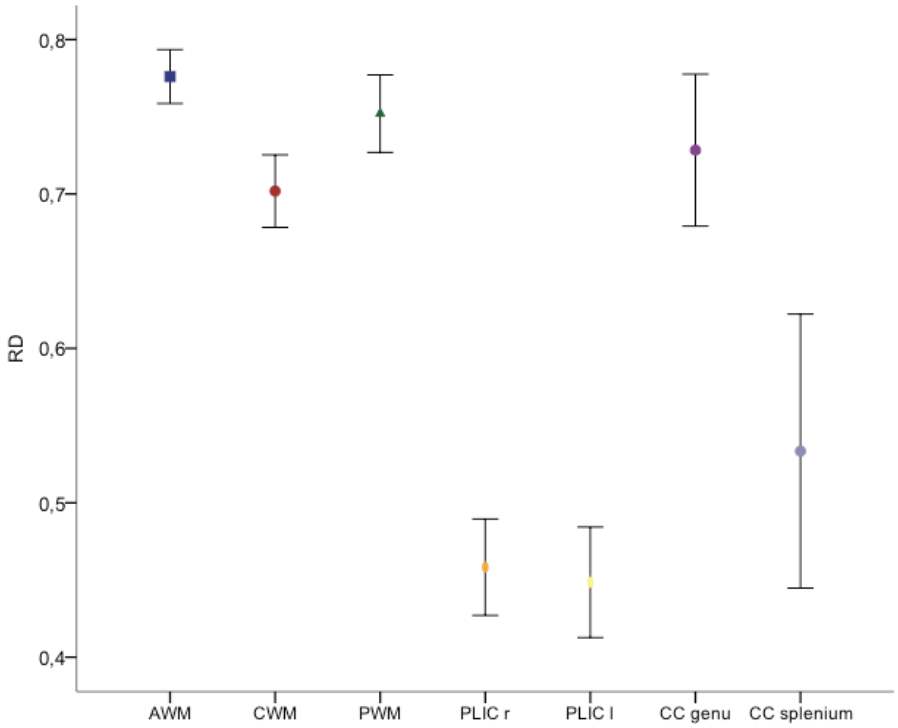


Fig.3.31c. Mean and standard deviations (error bars) of RD in AWM, CWM, PWM, PLIC bilaterally and genu and splenium of the corpus callosum (CC)

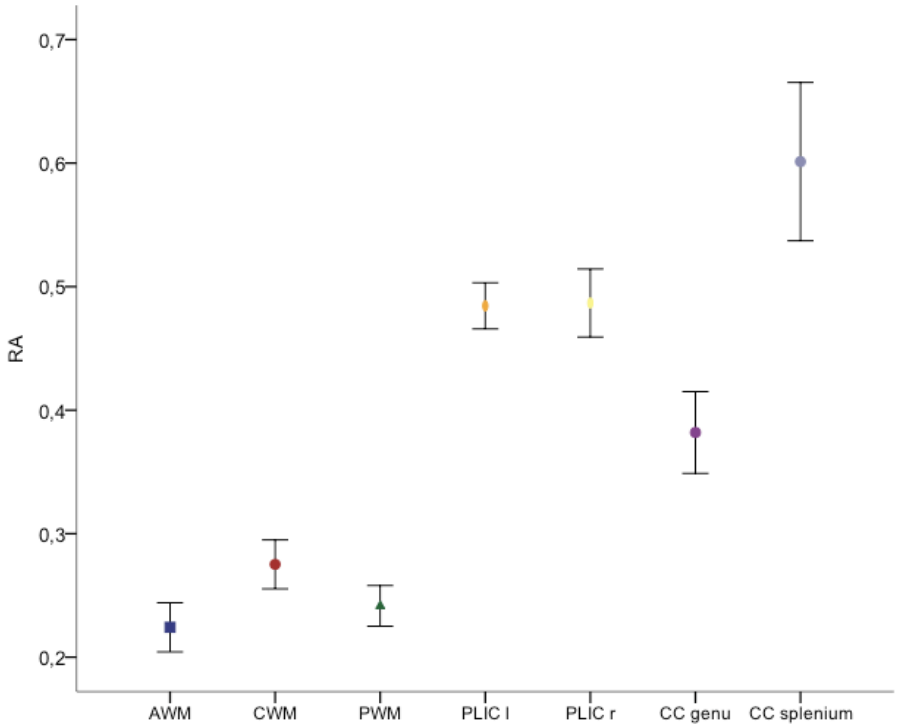


Fig.3.31d. Mean and standard deviations (error bars) of RA in AWM, CWM, PWM, PLIC bilaterally and genu and splenium of the corpus callosum (CC)

Of all measured RD ROIs, RD was highest in the CSO AWM and lowest in the left PLIC. Within CSO WM, RD was highest in AWM; the difference compared to CWM was significant (Fig.3.31c). CSO PWM RD was significantly higher than CSO CWM

RD, but no difference was found between CSO AWM and CSO PWM RD. RD was higher in right than in the left PLIC. RD of the genu of the CC was significantly higher than the splenium of the CC (Fig.3.31c).

The highest RA was found in the splenium of the CC, significantly higher than in the genu of the CC (*p<0.01*) (Table 64, Fig.3.31d). Within CSO WM regions, CWM RA was significantly higher than AMW or PWM RA. PWM was significantly higher than AWM RA. No difference was found between right and left PLIC RA.

3.8.2. Correlation between FA, EV1, RD and RA with gestational age

There was no correlation between AWM FA, CWM FA, PWM and PLIC FA with gestational age at birth. Splenium CC FA correlated closely with gestational age at birth (*p<0.01*, regression coefficient (95%CI) 0.03 (0.015 to 0.045)). Genu CC FA showed a trend to correlate with gestational age at birth (*p=0.05*).

Splenium EV1 showed a negative correlation with gestational age at birth (*p=0.03*, regression coefficient (95%CI) -0.02 (-0.045 to -0.03)), but no other EV1 value showed correlation with gestational age at birth.

None of the measured RD values correlated with gestational age. There was a trend of splenium RD to correlate negatively with corrected age at MRI (*p=0.05*, regression coefficient (95%CI) -0.03 (-0.06 to 0.001)).

No correlation was found between AWM RA, CWM RA, PWM RA, right or left PLIC, genu or splenium of the CC with gestational age at birth. There seemed to be a trend for correlation between left PLIC and gestational age at birth (p=0.07) and splenium of the CC and gestational age at birth (p=0.06). When corrected for corrected age at MRI both splenium RA and left PLIC RA correlated significantly with gestational age at birth.

3.8.3. Correlation between FA, EV1, RD and RA with corrected gestational age at scanning

No correlation was found between any EV1, RD or FA measurements and corrected gestational age at MRI. Left PLIC RA correlated well with corrected gestational age at MRI (*p<0.01*, regression coefficient (95%CI) 0.016 (0.005 to 0.027)).

3.8.4. WM FA, EV1, RD and RA in preterm infants with and without cystic lesions

CWM FA was significantly higher in preterm infants without cystic lesions (n=49) than in preterm infants with cystic lesions (n=12) (0.31 vs 0.27). No other WM FA showed any difference between preterm infants with and preterm infants without cystic lesions. No significant differences were found in CSO WM EV1 between preterm infants with and without cystic lesions. Significant differences were found in genu RA and genu RD between preterm infants with and without cystic lesions (*p=0.03* and *p= 0.02* respectively).

3.8.5. WM FA, EV1, RD and RA in preterm infants with and without DEHSI

CWM FA was significantly higher in preterm infants without DEHSI (n=51) than in preterm infants with DEHSI (n=11) (0.34 vs 0.30, p=0.03). No other FA, EV1, RD or RA differences between preterm infants with and without DEHSI could be found. When infants with cystic lesions were excluded, mean AWM RD became significantly higher in preterm infants with DEHSI (n=37) than in preterm infants without DEHSI (n=11) but no other FA, EV1, RD or RA difference became significant.

3.8.6. Correlation between clinical data and FA, EV1, RD and RA

No correlation was found between any clinical data and CWM FA and PWM FA. AWM FA correlated significantly with oxygen requirement at 28 days. On univariate analysis splenium CC FA correlated with oxygen requirement at 28 days, oxygen requirement at corrected gestational age of 36 weeks, postnatal hydrocortisone administration, gestational age at birth and birth weight. On multiple linear regression, none of the above mentioned clinical parameters was significant. Mean genu CC FA correlated with gestational age at birth, birth weight, oxygen requirement at 28 days, oxygen requirement at corrected gestational age of 36 weeks and PDA on univariate analysis. On multiple linear regression, oxygen requirement at 28 days remained significantly correlated with mean genu CC FA.

Mean CSO WM EV1 did not correlate with any clinical data. On univariate analysis mean splenium CC EV1 correlated with oxygen requirement at 28 days, oxygen requirement at corrected gestational age of 36 weeks and gestational age at birth on univariate analysis, but on multiple linear regression none was significant. No correlation was found between any clinical data and mean genu CC EV1.

None of the mean WM RD correlated with any clinical parameters.

Mean AWM RA and mean splenium CC RA correlated with oxygen requirement at 28 days. Mean splenium CC RA correlated with birth weight, gestational age at birth and oxygen requirement at corrected gestational age of 36 weeks. Mean CWM RA correlated with chorioamnionitis with funisitis. For splenium CC RA, on multiple linear regression splenium CC RA only correlated significantly with birth weight.

3.8.7. Correlation between FA, EV1, RD and RA and neurodevelopmental outcome

3.8.7.1. At one year

Mean AWM FA correlated significantly with fine motor scaled score (*p=0.04*). Mean CWM FA correlated with composite motor score, fine motor scaled score and composite cognitive score. Mean splenium FA correlated significantly with all outcome measures except for gross and fine motor scaled score. Mean genu FA correlated with receptive communication scaled score (p=0.04, R^2 0.09).

Mean PWM EV1 correlated well with composite language score, no other mean WM EV1 correlated with outcome measures.

Mean splenium RD did correlate significantly with composite cognitive and language scores and with expressive communication scaled scores. Mean CSO WM RD and mean PLIC RD did not correlate with any outcome measures.

Mean splenium CC RA correlated significantly with composite cognitive score, composite motor score, composite language score, receptive (*p=0.03*) and expressive communication (*p=0.03*) scaled score, but not with fine or motor scaled scores. No CSO WM RA correlated with outcome measures.

3.8.7.2. At two years

3.8.7.2.1. Univariate linear regression

3.8.7.2.1.1. In all preterm infants

There was a trend between mean PWM FA and composite motor scores (*p= 0.06*), between mean PWM FA and fine motor scaled score (*p=0.07*) and between AWM FA/CWM FA and gross motor scaled scores (*p=0.07*). No correlations were found between composite cognitive scores, composite language scores, receptive communication scaled, expressive communication scaled scores and any measured FA.

No correlations were found between composite cognitive scores and any EV1 measurements. Splenium CC EV1 correlated with composite language scores and receptive communication scaled scores.

Mean splenium and mean genu CC RD correlated with composite language scores. There was a trend between splenium CC RD, mean genu CC RD and receptive and expressive communication scaled scores. No correlations were found between any CSO RD and outcome measures.

Significant correlations were found between composite language scores/ expressive communication scaled scores and mean AWM RA.

3.8.7.2.1.2. In preterm infants without cystic lesions

No correlations could be found between measured WM FA and any outcome measures in preterm infants without cystic lesions.

Mean genu EV1 correlated with composite cognitive score, but no other correlation could be found between EV1 measurements and composite cognitive scores. Mean AWM EV1, mean CWM EV1 and mean PWM EV1 correlated with composite language scores and with receptive communication scaled scores. Expressive communication scaled scores correlated with mean AWM EV1, mean PWM EV1, mean right PLIC EV1 and mean genu CC EV1. No correlation was found between motor outcome and any EV1 measurements.

No correlation was found between motor outcome and any measured WM RD. However, mean genu CC RD and mean PLIC RD correlated with composite language scores. Mean CWM RD and mean PLIC RD correlated with receptive communication scaled scores and mean CWM RD correlated with expressive communication scaled scores.

No correlations could be found between measured WM RA and any outcome measures.

3.8.7.2.2. Stepwise linear regression

After stepwise linear regression was performed with all MR variables, all WM FA and BW/GA, FA in the splenium of the CC correlated with cognitive outcome at 2 years (Table 3.68) (Fig.3.32).

Composite cognitive score	***p value***	**Regression coefficient (95% CI)**
FA in splenium of CC	*0.047*	18.8 (0.3 to 37.3)
IVH	*<0.01*	-13.6 (-22.1 to -5.3)

Table 3.68. Stepwise linear regression for WM FA, MR variables, GA/BW with cognitive outcome

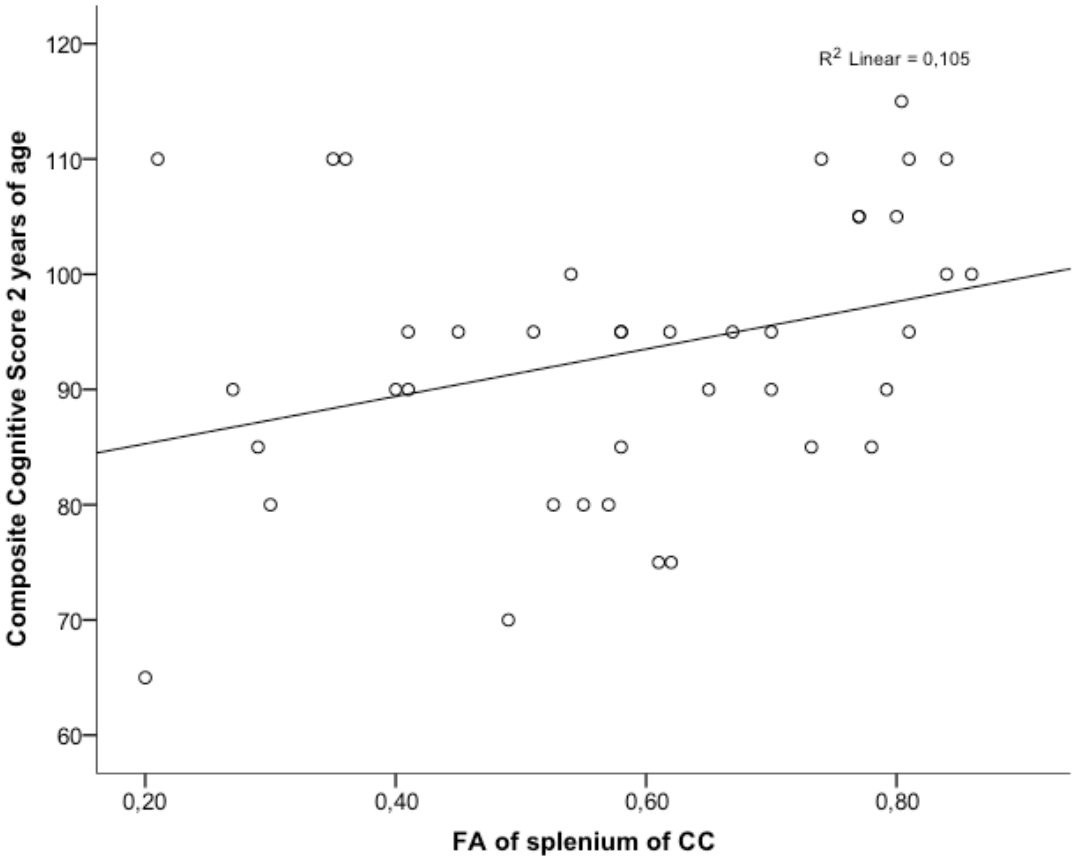


Fig.3.32. Scatterplot showing correlation between splenium FA and cognitive outcome at two years of age in all preterm infants

No FA measurement correlated with composite motor, gross and fine motor outcome. Splenium FA correlated with composite language and receptive language outcome but not with expressive language outcome (Table 3.68).

Composite language score	***p value***	**Regression coefficient (95% CI)**
FA in splenium of CC	*<0.01*	39.0 (12.0 to 66.0)
Receptive language scores	***p value***	**Regression coefficient (95% CI)**
FA in splenium of CC	*0.01*	6.4 (1.6 to 11.2)

Table 3.69. Stepwise linear regression for WM FA, MR variables, GA/BW with language outcome

Splenium EV1 and mean CWM EV1 correlated with composite cognitive outcome with adjustment for all MR variables, all WM FA and BW/GA (Table 3.70). No EV1 measurement correlated with composite motor outcome. EV1 in the splenium of the CC correlated with composite language and receptive language outcome (Table 3.71).

Composite cognitive score	***p value***	**Regression coefficient (95% CI)**
EV1 in splenium of CC	*<0.01*	-23.7 (-37.0 to -10.3)
Mean CWM EV1	*<0.01*	29.9 (5.5 to 54.3)
IVH	*<0.01*	-14.9 (-22.5 to -7.3)

Table 3.70. Stepwise linear regression for WM EV1, MR variables, GA/BW with cognitive outcome

Composite language score	***p value***	**Regression coefficient (95% CI)**
EV1 in splenium of CC	*<0.01*	-30.0 (-49.0 to 11.1)
Small CC	*<0.01*	-15.5 (-26.5 to -4.4)
Receptive language scores	***p value***	**Regression coefficient (95% CI)**
EV1 in splenium of CC	*<0.01*	-4.8 (-8.3 to 1.5)
HPI	*0.03*	-4.2 (-7.9 to -0.4)

Table 3.71 Stepwise linear regression for WM EV1, MR variables, GA/BW with language outcome

Splenium RD correlated with composite cognitive outcome with adjustment for all MR variables, all WM RD and BW/GA (Table 3.72).

Composite cognitive score	***p value***	**Regression coefficient (95% CI)**
RD in splenium of CC	*0.014*	-11.5 (-20.5 to -2.5)
IVH	*<0.01*	-14.3 (-20.5 to -2.5)

Table 3.72. Stepwise linear regression for WM RD, MR variables, GA/BW with cognitive outcome

No RD measurement correlated with motor outcome. RD of the splenium of the CC correlated with composite language scores (p=0.012), but not with the language subscales.

RA in the splenium of the CC correlated after stepwise linear regression with composite cognitive score (p=0.01), composite language scores (p=0.01) and receptive language scores (p=0.01). No correlation was found between any motor outcome and WM RA.

Short summary:

Only CWM FA was significantly different between preterm infants with or without cystic lesions and with or without DEHSI. Splenium FA correlated with cognitive and language outcome, no correlation was found between motor outcome and any FA value.

3.9. Correlation between T2 and ADC measurements

Linear regression analysis showed good correlation between mean WM/GM ADC and mean WM/GM T2 measured in the same ROIs (Table 3.72, Fig.3.31a): significant correlation could be found in all WM and GM ROIs. No correlation was found between mean brainstem T2 and mean brainstem ADC. Correlations remained significant after excluding preterm infants with cystic lesions (Fig.3.31b).

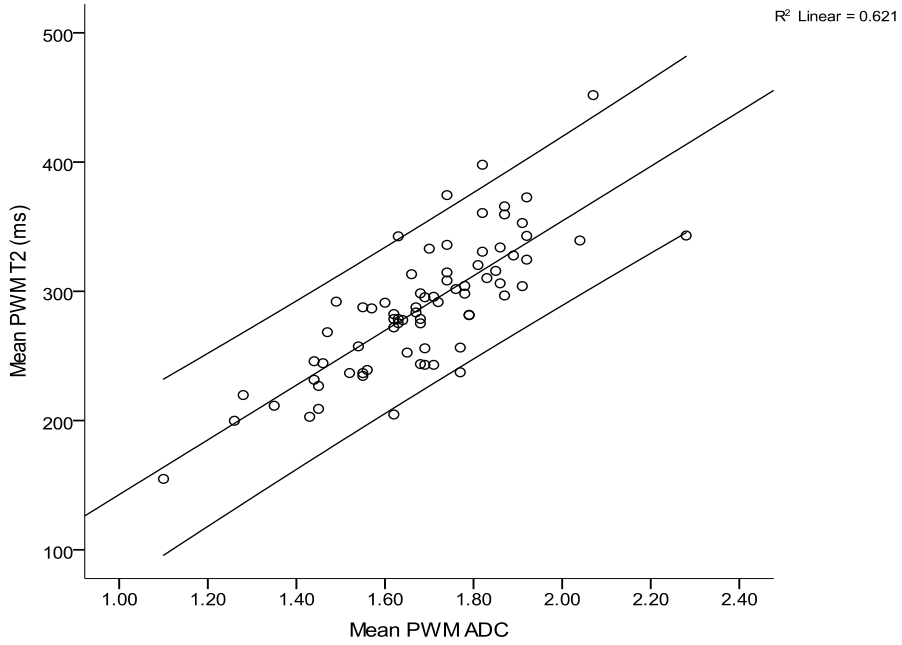


Fig. 3.33a. Scatterplot showing good correlation between mean PWM T2 and mean PWM ADC in all preterm infants.. Inner line represents the linear fit, outer lines CI 95%

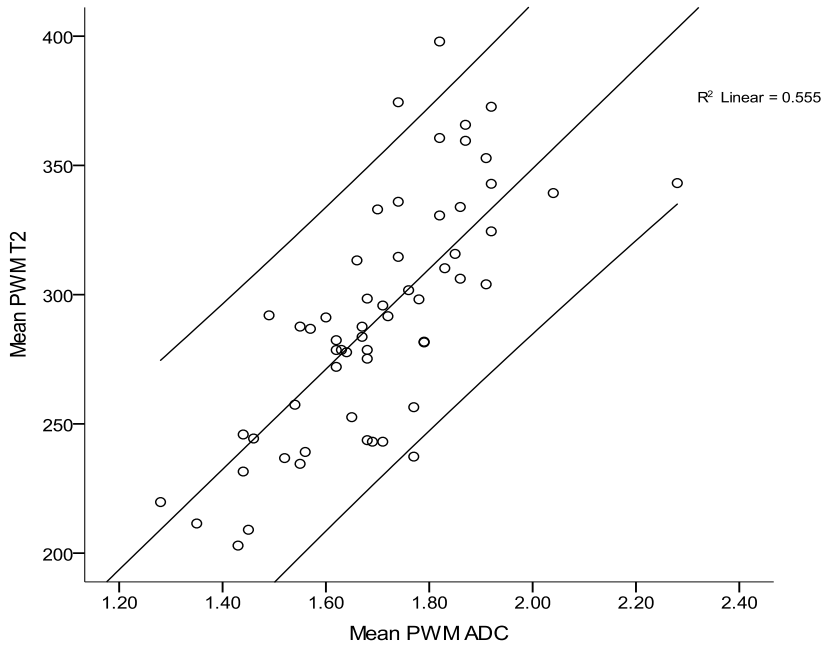


Fig. 3.33b. Scatterplot showing correlation between mean PWM T2 and mean PWM ADC in preterm infants without cystic lesions. Inner line represents the linear fit, outer lines CI 95%

T2 of FA equivalent ROIs	*p value*	Regression coefficient (95% CI)
AWM	<0.01	131.0 (87.4-174.6)
CWM	<0.01	155.4 (125.8-185.1)
PWM	<0.01	211.6 (172.8-250.5)
AWM pv	<0.01	166.1 (115.1-217.2)
PWM uniform	<0.01	208.0 (168.2-247.8)
Caudate	<0.01	68.9 (32.4-105.5)
Thalamus	<0.01	103.3 (67.5-139.0)
vl Thalamus	<0.01	166.9 (128.9-204.9)
Brainstem	0.2	28.4 (-14.4-71.1)
Cerebellum	<0.01	116.1 (81.9-150.4)

Table 3.73. Linear correlation between WM/GM ADC and WM/GM T2. T2 and ADC were measured in the same ROIs of all preterm infants.

3.10. Correlation between T2 and FA/eigenvalue measurements

Linear regression analysis showed good correlation between all CSO WM T2 values and CSO WM FA (Table 3.73). No correlation was found between AWM T2 and AWM EV1, CWM T2 and CWM EV1 respectively, however good correlation was found between PWM EV1 and PWM T2. WM T2 correlated well with WM RD and WM RA, apart from AWM T2 and AWM RA. These correlations remained significant after adjustment for gestational age at birth and corrected gestational age at MRI.

T2 of FA equivalent ROIs	***p value***	**Regression coefficient (95% CI)**
AWM	0.01	-208.1 (-370.4 to -45.8)
CWM	<0.01	-286.6 (-420.9 to -152.2)
PWM	<0.01	-331.7 (-519.3 to -144.2)
T2 of EV1 equivalent ROIs	***p value***	**Regression coefficient (95% CI)**
AWM	0.72	9.0 (-41.8 to 59.9)
CWM	0.74	10.0 (-49.9 to 70.2)
PWM	0.03	83.8 (7.2 to 160.5)
T2 of RD equivalent ROIs	***p value***	**Regression coefficient (95% CI)**
AWM	<0.01	129.4 (43.5 to 215.4)
CWM	<0.01	250.9 (163.5 to 338.4)
PWM	<0.01	278.7 (169.8 to 387.7)
T2 of RA equivalent ROIs	***p value***	**Regression coefficient (95% CI)**
AWM	0.08	-124.7 (-265.9 to 16.5)
CWM	<0.01	-220.5 (-355.9 to -85.1)
PWM	<0.01	-335.4 (-516.8 to -153.8)

Table 3.73. Linear correlation between WM FA/EV1/RD/RA and WM T2. T2 and FA/EV1/RD/RA were measured in the same ROIs of all preterm infants

3.11. Correlation between ADC and FA/eigenvalue measurements

Linear regression analysis showed good correlation between all measured WM ADC and WM RD (Table 3.74). AWM EV1 and CWM EV1 showed no correlation with AWM ADC and CWM ADC respectively, however PWM EV1 correlated well with PWM ADC. The same pattern of correlation was found between WM ADC and WM RA.

The opposite pattern of correlation was found for WM ADC and WM FA; AWM and CWM values correlated well whereas there was no correlation between PWM ADC and PWM FA.

ADC of FA equivalent ROIs	***p value***	**Regression coefficient (95% CI)**
AWM	<0.01	-1.3 (-2.2 to -0.7)
CWM	<0.01	-0.9 (-1.7 to -0.2)
PWM	0.05	-0.6 (-1.4 to 0.02)
ADC of EV1 equivalent ROIs	***p value***	**Regression coefficient (95% CI)**
AWM	0.5	-0.06 (-0.2 to 0.1)
CWM	0.5	0.08 (-0.1 to 0.3)
PWM	<0.01	0.45 (0.2 to 0.6)
ADC of RD equivalent ROIs	***p value***	**Regression coefficient (95% CI)**
AWM	<0.01	0.6 (0.3 to 0.9)
CWM	<0.01	1.0 (0.5 to 1.5)
PWM	<0.01	0.8 (0.4 to 1.2)
ADC of RA equivalent ROIs	***p value***	**Regression coefficient (95% CI)**
AWM	0.02	-0.5 (-0.9 to -0.06)
CWM	0.06	-0.6 (-1.2 to 0.4)
PWM	<0.01	-0.9 (-1.5 to -0.3)

Table 3.74. Linear correlation between WM FA/EV1/RD/RA and WM ADC. ADC and FA/EV1/RD/RA were measured in the same ROIs of all preterm infants

Chapter 4

4. Discussion

T2 relaxometry

White matter T2

In this study WM T2 values were prolonged in preterm infants compared to healthy controls. Interestingly when preterm infants were grouped into those with and those without DEHSI, preterm infants without DEHSI had similar WM T2 to control infants. This is consistent with the findings of Counsell et al (Counsell et al. 2003) and Ferrie et al (Ferrie et al. 1999) who reported similar T2 in small numbers of preterm infants at term equivalent age with normal appearing WM on conventional MRI. In addition, we were able show to that WM T2 values of preterm infants with DEHSI were significantly longer compared to controls infants and compared to preterm infants without DEHSI.

Brain maturation is associated with T2 shortening. The major T2 determinant is the nature and frequency of interactions between water protons and tissue macromolecules (Bloembergen EM 1948). With the long TEs used in newborn infants, and in the absence of a multi-exponential model, our measured WM T2 values can be principally attributed to axonal and extracellular water (Whittall et al. 1997; Baratti et al. 1999). Increased WM T2 in preterm infants at term equivalent age in this study could result from a change in the water-macromolecule ratio due to either delayed maturation or to loss of tissue integrity resulting in increased free water. Loss of tissue integrity is described in later stages of diffuse WM injury where areas of injury contain numerous reactive astrocytes (diffuse gliosis) (Leviton and Gilles 1984; Haynes et al. 2003; Back 2006) compared to early stages of injury where diffuse WM damage is characterised by the presence of numerous reactive microglia in the periventricular WM (Kinney and Back 1998).

Regional WM T2 variation

Regional variation in increased CSO WM T2 with most marked increases in the CSO PWM, particularly in infants with DEHSI, was observed. Williams et al reported T2 measurements at 3 Tesla in 4 preterm infants at term equivalent age with periventricular leukomalacia (Williams et al. 2005); T2 in these infants was similar to that in infants with DEHSI in this study. Although only 4 infants were studied, it is interesting to note that similar to our findings, T2 was longest in PWM (Williams et al. 2005). In this study, CSO T2 was the shortest of all measured WM T2 values in preterm and control infants. Counsell et al however found no regional WM T2 variation at term equivalent age in preterm infants; but before term, CWM T2 was

increased compared to frontal or occipital white matter (Counsell et al. 2003). We found shorter CWM T2 than in any other CSO WM regions also in term controls. Indeed, one would expect shorter CWM T2 as myelin (associated with T2 shortening) should be present in the central part of the CSO around term and hence, T2 would be shorter (Kinney et al. 1988; Van der Knaap 2005).

Deep WM myelinates in a dorsal to rostral direction, with deep occipital WM maturing first and frontal WM last (Flechsig 1920; Barkovich et al. 1988; Kinney et al. 1988; Van der Knaap 2005). Experimental studies have suggested that the vulnerability to injury of periventricular WM in the preterm brain relates to both timing of appearance and regional distribution of susceptible oligodendrocyte progenitors (Back et al. 2001; Riddle et al. 2006; Back et al. 2007). Preterm WM injury is characterised by a 50% to 90% depletion of pre-myelinating oligodendroglia with relative sparing of other axonal and glial elements (Back et al. 2007). It is possible that regional WM T2 variation could be due to the presence of more late progenitor oligodendrocytes in PWM compared to other WM regions at a critical period when many injurious processes occur such as oxidative stress and hypoxia-ischaemia. This would lead to localised disruption of the oligodendrocyte lineage, affecting PWM more than other WM regions, and increased T2 may relate to regional variation in reactive astrogliosis.

Grey matter T2

All GM T2 values were significantly shorter compared to any WM T2 values measured in this study. This is consistent with previous reports (Baierl et al. 1988; Ferrie et al. 1999; Thornton et al. 1999; Ding et al. 2004). GM T2 values in those studies are comparable to GM T2 values of the control infants in the present study. GM T2 values were similar in preterm infants without DEHSI and control infants whereas preterm infants with DEHSI had significantly longer GM T2 than control infants consistent with WM T2 findings. This is of interest as in many preterm infants volumetric studies have shown deep grey matter volume abnormalities especially in those infants with concurrent WM abnormalities (Boardman et al. 2006). Hence, prolonged GM T2 values could reflect injury to deep grey matter.

Regional variation of GM T2

Regional GM T2 variation was present: caudate T2 was longest of all measured GM T2 values in preterm and control infants. Thornton et al measured T2 only in the thalami; hence, no comparable caudate T2 data are available from that study (Thornton et al. 1999). In the study by Ding et al, caudate and thalamic T2 were measured in 3 weeks old newborn infants: caudate T2 was longer than thalamic T2,

consistent with our findings (Ding et al. 2004). The regional GM T2 variation could be explained by either the myelination progress at term equivalent age as ventral lateral thalami are myelinating at this stage although not the caudate or in preterm infants, it could also be explained by injury resulting in loss of tissue integrity. Whether there is a primary injury due to the close location of the caudate to the germinal matrix where haemorrhages are frequent and the risk for injury to the caudate is high or whether the caudate abnormalities are secondary to maturational trophic disturbances as proposed by Volpe et al, cannot be said. However, infants with GMH-IVH had similar T2 values to those without GMH-IVH, hence, a maturational trophic process might be more likely.

Correlation of T2 with outcome

At one year corrected age PWM T2 correlated significantly with cognitive, motor and receptive language outcome in preterm infants without cystic lesions, even after correction for birth weight, cerebellar haemorrhages, intraventricular haemorrhages and DEHSI. At two years of age, mean AWM CSO T2, mean PWM CSO T2, periventricular AWM T2, uniform AWM T2 and uniform PWM T2 correlated significantly with cognitive outcome on linear regression, even after correction for cystic white matter lesions, birth weight, cerebellar haemorrhages, intraventricular haemorrhages and DEHSI. However, when all T2 values (GM and WM) and all MR variables were included into a stepwise linear regression then only mean pv AWM T2, HPI and IVH remained independent risk factors for cognitive outcome at two years of age. Caudate T2 was an independent risk factor for language, receptive and expressive language outcome and for motor outcome; and periventricular AWM T2 remained a significant risk factor for fine motor outcome on multiple linear regression. Thus prolonged frontal periventricular WM T2 and caudate T2 correlated significantly with neurodevelopmental outcome at two years in this cohort. No study to date has tried to correlate T2 measurements at term equivalent age with neurodevelopmental outcome in preterm infants. Periventricular frontal WM T2 was longest of all measured WM T2 values and PWM T2 CSO was longest of all CSO WM T2 values; these prolonged T2 values correlated with high ADC values in these regions. FA and eigenvalues were not measured in the periventricular frontal WM regions. However, one could speculate that as there was a correlation between prolonged CSO WM T2 and increased radial diffusivity in CSO WM, a similar correlation between T2 and radial diffusivity would apply for the WM at the level of the basal ganglia. Prolonged WM T2 and increased radial WM diffusivity could reflect abnormal tissue microstructure. Experimental data show that the developmental predilection of

periventricular white matter injury appears to relate to the regional distribution of susceptible oligodendrocyte progenitors, which explains the vulnerability of the frontal periventricular and posterior white matter (Back et al. 2007). Volumetric MR studies in preterm children and adolescents have shown volume reduction in the frontal and occipital lobes, which is consistent with earlier injury in this brain region (Thompson et al. 2007) and the quantitative MR findings in this study might describe such injury.
In the present study, mean caudate T2 correlated significantly with composite language, receptive and expressive communication and motor outcome on stepwise linear regression. Of note is that caudate T2 was significantly longer compared to all other measured deep grey matter structures. How can language and motor problems relate to prolonged caudate T2? Anatomical circuits directly associated with the caudate are the associative, lateral orbitofrontal, and the oculomotor circuits. The associative circuit is believed to regulate executive functions by unifying cognitive processes such as attention, planning and decision-making. Abnormalities in the lateral orbitofrontal circuit result in short memory deficits (Ni et al. 2011). Although the caudate is not considered part of the motor system, it contributes to working memory in the planning and selection of motor sequences (Dagher et al. 1999). Hence, the caudate participates in sensorimotor, cognitive and executive functions reflecting its role in functional networks. Interestingly, small caudate volumes have been described in boys with ADHD (Castellanos et al. 1994) and in children with autism (Hrdlicka et al. 2005). In preterm children at 7 and 15 years respectively caudate volume has been associated with cognitive outcome in children born preterm (Abernethy et al. 2004; Isaacs et al. 2008). It will be interesting to investigate this cohort for executive function deficits and to investigate whether prolonged caudate T2 is associated with such deficits.

Why was there a difference in correlation of T2 values and outcome between one and two years? This might be explained by the fact that composite cognitive scores, gross and fine motor outcome were significantly lower at two years than at one year of age on paired sample t test. FU rate at one year was 76.3%; 61 of 80 infants were seen. It is known from FU studies that assessment at one year of age underestimates the real outcome as one year is an early age for cognitive assessment. At two years of age, only 54 (68%) infants attended a neurodevelopmental assessment, which is a limitation of the study. Four more families moved abroad between one and two years assessments and sixteen families compared to eleven at one year did not attend the FU appointment despite repeated invitation letters and several phone calls with the offer of home visits. Although low,

this FU rate is comparable to other preterm cohorts within London (Boardman et al.). Forty-nine infants were seen at both appointments. The majority (35/49) of those parents had a higher education. There were no significant differences in outcome measures between those who were seen at both appointments and those who were seen only at one year of age; but those who were seen at both appointments, were younger and smaller. When infants with 2 year FU were compared with those without 2 year FU, there was no significant difference in known clinical risk factors for outcome other than gestational age and birth weight. Hence, when correlation between outcome measures and MR variables were done, correction for gestational age and birth weight was always performed. A sample size calculation

Only one study was found which examined the correlation between T2 values and outcome: Abernethy et al studied WM T2 in seven years old children born preterm. They could not show any correlation between WM T2 and IQ (Abernethy et al. 2003). Maybe WM and GM T2 values might be more predictive when measured at term equivalent age instead of at later age or maybe, if Abernethy et al would have covered more brain regions, correlation could have been found between T2 values and outcome.

Conclusions

WM and GM T2 values showed significant differences between preterm subgroups and between control and preterm infants with regional variation in preterm infants. T2 values correlated well with cognitive, motor and language outcome at two years of age, even after multiple corrections for clinical and MR variables. No study so far has shown any correlation between T2 values at term equivalent age and later neurodevelopmental outcome. T2 might be a promising MR biomarker as T2 seems to fulfil all criteria a biomarker should have as defined by the NIH (NIH Definitions Workgroup 2000).

ADC measurements

White matter ADC measurements

Similar to T2 measurements, WM ADC was significantly higher in preterm infants than in control infants, apart from CSO CWM ADC. Preterm infants with DEHSI had significantly higher WM ADC compared to preterm infants without DEHSI, apart from CSO CWM and periventricular AWM ADC. Preterm infants without DEHSI had similar WM ADC as the control infants. In principle, WM ADC showed a similar

pattern to WM T2 measurements although T2 values were more consistently different between preterm and control infants and between preterm groups.

Counsell et al reported significant CSO WM ADC differences between preterm infants with and without DEHSI (Counsell et al. 2003); their initial findings were confirmed in a subsequent study including control infants (Counsell et al. 2006). A recent study could not show any differences of WM ADC between preterm infants with and those without DEHSI; however only 43.5% of the preterm infants had DEHSI (Hart et al.) which is in contrast to a previously reported incidence of up to 75% (Maalouf et al. 1999). DEHSI is based on visual assessment, hence, the grouping of infants could be different depending on the radiologist/neonatologist assessing the images and so correlation might be different. This shows the importance of a reliable quantitative MR measurement to assess WM integrity.

Regional WM ADC variation

Regional WM ADC variability was seen at the level of CSO and at the level of basal ganglia. Highest WM ADC values were measured in the PWM CSO and at the level of the basal ganglia highest WM ADC was found in the frontal WM. No regional WM ADC was found in control infants. This is in contrast to previous studies that found no regional variation between FWM, CWM and PWM CSO ADC (Neil et al. 1998; Counsell et al. 2003). Regional WM ADC variation was consistent with regional CSO WM T2 variation in this study.

GM ADC measurements and regional variability

Mean putamen ADC and mean brainstem T2 were significantly higher in preterm infants than in control infants. There were no significant differences in mean GM ADC between preterm infants with and without DEHSI even after exclusion of preterm infants with cystic lesions. This is in contrast to T2 measurements, which showed significant GM T2 differences between preterm infants with and without DEHSI. Caudate ADC was highest in all infants' subgroups; apart from this no regional pattern of ADC was found. Hence, it seems that T2 measurements provide more regional information than ADC measurements.

Correlation of ADC with outcome

At two years of age, caudate ADC correlated significantly with language and expressive language outcome, and periventricular frontal WM ADC correlated significantly with receptive language outcome after stepwise linear regression was

performed, with adjustment for all MR variables, all quantitative ADC measurements and BW/GA. No ADC measurement correlated with cognitive or motor outcome at 2 years.

Hence, the findings by Krishnan et al showing that higher ADC values were related to lower DQ at two years could not be confirmed in this study (Krishnan et al. 2007) as no WM or GM ADC correlated with cognitive outcome on multiple linear regression. Krishnan et al measured ADC in the entire CSO whereas we measured six ROIs within the WM of the CSO: that might explain the lack of correlation in this study. However, in the study by Krishnan et al, ADC was only corrected for postmenstrual age and not for any other clinical or MR variables (Krishnan et al. 2007). However, in this study WM T2 measurements correlated with cognitive outcome; therefore, maybe ADC might not be as predictive as T2 for cognitive outcome.

Conclusions

In the present study, T2 exhibited more differences between preterm and control infants and between preterm infants with and without DEHSI than ADC. T2 showed a stronger correlation with later outcome than ADC. Hence, T2 might be more sensitive in the evaluation of WM injury than ADC in the preterm population due to the nature of preterm brain injury, resulting in better prediction of outcome of T2 than ADC.

FA/eigenvalue measurements

WM FA/eigenvalue measurements

CWM FA was significantly higher in preterm infants without DEHSI than in preterm infants with DEHSI. No other FA, EV1, RD or RA differences between preterm infants with and without DEHSI could be found.

Regional variability of FA/eigenvalue measurements

Highest FA of all measured ROIs was found in the splenium of the CC and lowest FA in CSO AWM. Regional FA variability was found within CSO WM regions and within the corpus callosum regions; CSO CWM FA was significantly higher than AWM FA and PWM FA. FA was significantly higher in the splenium of the CC than in the genu of the CC. This regional variability can be explained by the progress of myelination.

FA/eigenvalue measurements and outcome

In this study, splenium FA correlated with cognitive, language and receptive communication outcome at two years of age on stepwise linear regression. There are only a few studies in which neonatal FA has been correlated with later outcome

(Arzoumanian et al. 2003; Drobyshevsky et al. 2007; Counsell et al. 2008; Rose et al. 2009). Three studies have correlated neonatal lower PLIC FA with motor outcome (Arzoumanian et al. 2003; Drobyshevsky et al. 2007; Rose et al. 2009) and one study correlated FA of parts of the CC with developmental quotient at two years of age (Counsell et al. 2008).

Many studies have shown that FA correlates with gestational age and can be used to study brain maturation in preterm infants as anisotropy increases with increasing age (Huppi and Inder 2001). A recent study showed that gestational age <26 weeks had an effect on the change in white matter fractional anisotropy; however this effect was eliminated by neonatal co-morbidities such as the presence of a PDA, need for mechanical ventilation and NEC (Bonifacio et al. 2011). The authors suggest that low gestational age itself is not a strong determinant of brain development as measured serially by DTI. In this study, only splenium CC FA correlated with gestational age at birth. When all MR variables (including cPVL, HPI, IVH, cerebellar haemorrhages, PWML, DEHSI) were added into a stepwise regression, then GA remained an independent risk factor for splenium FA. When clinical parameters such as the presence of PDA, NEC and days of mechanical ventilation were added into the stepwise linear regression, then only days of mechanical ventilation remained an independent risk factor for splenium FA which is consistent with the study by Bonifacio et al (Bonifacio et al. 2011).

We were able show that splenium FA remained an independent risk factor for cognitive and language outcome on stepwise binary logistic regression. Hasegawa et al performed a DTI study in preterm infants without apparent white matter injury at term equivalent age. They divided the preterm infants according to their gestational age at birth into three groups. FA in the splenium of the corpus callosum decreased linearly with decreasing gestational age. They suggested that the development of the posterior corpus callosum was affected by prematurity itself (Hasegawa et al. 2011); which is what we found as well when clinical variables were not corrected for. Counsell et al assessed the relationship between WM FA using TBSS at term equivalent age and the developmental quotient (DQ) at two years of age. They found that DQ was linearly related to parts of the corpus callosum (Counsell et al. 2008). In the present study, ROIs analysis showed that FA, EV1 and RA in the splenium of the CC correlated even after stepwise linear regression (all MR variables, GA/BW) with composite cognitive outcome at 2 years: lower FA, EV1 and RA in the splenium were associated with lower composite cognitive scores at two years of age. Radial

diffusivity did not correlate with cognitive outcome. Changes in radial diffusivity but not in axial diffusivity are seen in regions with high anisotropy such as the posterior part of the corpus callosum.

It seems that altered microstructure in the CC might have resulted in abnormal size as small corpus callosum was associated with splenium FA and EV1 and with genu RA and genu RD. These quantitative DTI results are important as they are independent risk factors for outcome and they might explain the abnormal callosal size. The size of the corpus callosum in this study was not assessed by volumetric studies; hence, to confirm these results quantitatively, volumetric MR analysis should be done. Automated FA analysis such as TBSS could not be done in this cohort as only 6 directions were acquired. Maybe FA evaluation by TBSS would have shown more WM regions correlating with outcome as shown in the study by Counsell et al (Counsell et al. 2008). However from the clinical side of view, as splenium FA, EV1 and RA are independent risk factors for cognitive outcome, they might be used to inform clinicians about risk for later cognitive impairment independent of any other MR findings.

These findings can be considered in the context of published DTI work on preterm children and adolescents. A MR study that looked at the differences in whole brain WM volumes and FA between preterm (neurologically normal with minimal changes on MR) and control children at 8.8 to 11.5 years showed that both WM volume and FA were independent risk factors for FSIQ in preterm infants after adjusting for gender, BW and GA (Yung et al. 2007). Kontis et al undertook a DTI study in young adults born preterm and in control adults. There were no correlations between FA/mean diffusivity (MD) and IQ in the control group. In the preterm group however, very preterm females had significantly higher MD in the genu of the corpus callosum than term female adults and higher genu MD was associated with lower IQ (Kontis et al. 2009). A DTI study of the CC in children born preterm showed higher FA values and lower MD values in adolescent born preterm compared to control adolescents (Fryer et al. 2008). Relationships were observed between microstructure of the CC (FA and MD) and performance on tests of visuospatial cognition, language, and psychomotor function. High values of FA in the CC correlated with optimal cognitive functioning (Fryer et al. 2008). It was suggested that WM coherence relates to maturation of certain cognitive abilities in adolescents and that there are some CC subregion specific brain-behaviour relationships, as only microstructure of the CC

splenium and not of any other parts of the CC related to language and psychomotor performance in healthy adolescents at 16 to 18 years of age (Fryer et al. 2008).

The degree of connectivity between hemispheres is thought to be an important factor in interhemispheric transfer and cooperation, with fiber size and density accounting for the regulation of the transfer (van der Knaap and van der Ham 2011). The degree of connectivity between the hemispheres can be reflected by the size of the corpus callosum (Aboitiz et al. 1992; Clarke and Zaidel 1994). Abnormal callosal size has been described in patients with schizophrenia (Bersani et al.), in children with autism (Hardan et al. 2000; Vidal et al. 2006; Hughes 2007; Freitag et al. 2009) and in ADHD (smaller splenium which connects to the parietal lone which supports sustained and divided attention) (Hutchinson et al. 2008).

Development of the corpus callosum takes place between 12 and 18 weeks (Volpe 2008). At 18 weeks pre-oligodendrocytes start to be present in the white matter, at the time when the period of the highest risk for PVL starts. The growth of the corpus callosum follows the expansion of the hemispheres, in a rostro-caudal and then dorso-ventral circular movement; hence myelination of the body and splenium of the corpus callosum occurs earlier than of the rostral part of the corpus callosum (Kinney et al. 1988). This might explain why the splenium of the corpus callosum is more affected than the genu or the rostrum of the corpus callosum as the splenium starts to myelinate earlier than the rest of the corpus callosum; hence, the splenium is more vulnerable.

The strength of our study is that the correlation between FA and outcome is shown at a very early stage and that FA correlated with cognitive, motor and language outcome. However, only splenium FA correlated with neurodevelopmental outcome on stepwise binary logistic regression. Therefore, one might consider performing automated FA analyses for conformation of these results before splenium FA can be used as MR biomarker for neuroprotective studies.

Qualitative MR techniques

In the present study, the frequencies of GMH-IVH, PWML, cerebellar haemorrhages, DEHSI, ventricular dilatation and increased extracerebral space are similar to other conventional MR studies (Maalouf et al. 1999; Miller et al. 2005; Dyet et al. 2006; Limperopoulos et al. 2009). HPI and cPVL were more frequent in this study compared to the literature. As there were so many infants with HPI or cPVL the

images were reviewed to ascertain the accuracy of the diagnosis. The seven (9%) cases with HPI were easily definable because of the porencephalic cysts; hence, there is no doubt on the correct diagnosis of HPI. All of these infants had antenatal steroids and gestational age ranged between 24 and 30 weeks. The only clinical parameter, which correlated with the presence of HPI on multiple linear regression, was inotropic use. This is in agreement with the results of Inder et al found who found on multiple linear regression an increased risk for moderate to severe WM abnormalities in infants with inotropic use (Inder et al. 2003). In this cohort, small corpus callosum and HPI were associated with inotropic use. At UCLH the guideline for blood pressure limits are mean blood pressure equal gestational age at birth; in 36% of the infants inotropes were used within the first few days after birth. Cystic WM lesions other than HPI were noted in 11(14%) infants. Of those, three (3.8%) had classical bilateral diffuse periventricular PVL. This seems high compared to the literature (van Haastert et al.). Infants with only small, focal, unilateral cysts within the periventricular WM were classified as cPVL. Infants with "classic cPVL" (n=3) and infants with focal cystic WM changes (n=8) did not differ in GA, BW and outcome measures at two years of age, however as a group of infants with cPVL they had lower scores on all outcome measures. There was no correlation between gestational age and cPVL: GA ranged between 23 and 32 weeks; all had antenatal steroids, neither chorioamnionitis, nor chorioamnionitis with funisitis, nor inotropic use, nor oxygen requirement at 28 days or 36 weeks correlated with the presence of cPVL. Worst pH within first 24 hours correlated with cPVL, however not worst lactate or worst BE within the first 24 hours. Unfortunately, no note of worst pCO_2 within the first 24 hours was made; however acidotic pH is associated with hypercarbia and not hypocarbia, which is a known risk factor for cPVL. If only the three infants with classic cPVL were analysed, then a correlation with chorioamnionitis was highly significant even after correction for PDA, NEC, CLD and inotropic use. However, as n=3, this has to be interpreted with caution and tested with a higher number.

Cognitive outcome correlated with the presence of IVH, cerebellar haemorrhage, HPI and cPVL. Infants with IVH on MR had a 5.1 times higher risk for cognitive outcome score below 85, infants with cPVL had a 7.9 higher risk and infants with cerebellar haemorrhage a 6.5 higher risk for cognitive outcome score below 85 respectively; and these risks were calculated after adjustment for gender, GA, birthweight, and other MR findings such as PWML, DEHSI, HPI, cPVL, small CC and cerebellar haemorrhage.

HPI, small CC and PWML were associated with motor outcome: HPI was an independent risk factor for composite motor scores below 85 on stepwise binary logistic regression and correlated with gross motor scaled scores. Small CC was an independent risk factor for gross motor outcome and PWML for motor outcome scores below 85; the risk for motor outcome below 85 was 4.2 higher in infants with PWML. PWML correlated with fine motor outcome on univariate linear regression. A recent DTI study of preterm infants without cystic WM lesions at term equivalent age showed that FA was lower in the corticospinal tract and in the cerebellar peduncles in infants with PWML compared to those infants without PWML (Bassi et al. 2011). Based on these findings the authors concluded that PWML is associated with altered WM microstructure (Bassi et al. 2011). In this study however, no significant differences in FA could be found in the PLIC between infants with or without PWML.

Small CC and DEHSI were independent risk factors for expressive language outcome after adjustment for GA, gender, birthweight, IVH, cerebellar haemorrhage, cPVL and HPI.

Conclusions

White matter injury including thinning of CC (Woodward et al. 2006), cerebellar haemorrhage (Limperopoulos et al. 2009), DEHSI (Maalouf et al. 1999; Dyet et al. 2006) and PWML (Miller et al. 2005) diagnosed with conventional MR at term equivalent age have been associated with neurodevelopmental impairment in preterm infants at 18 to 24 months of age. However, these studies did not perform stepwise logistic regression with other MR variables to find an independent MR risk factor for neurodevelopmental impairment. In this study, HPI, cPVL, IVH, cerebellar haemorrhage, small CC and PWML were independent risk factors for either cognitive or motor outcome. The only MR variables that correlated with language outcome were DEHSI and small CC.

Chapter 5

5. Conclusions

A biomarker has to be qualified by firm evidence that it can detect a particular effect; it must be objectively measured and evaluated to serve as a biological marker or surrogate endpoint. In the present study, either conventional MR, or quantitative MR measurements such as T2, ADC or FA could serve as biomarkers if they satisfy the required criteria.

What is the evidence in this study that conventional or qualitative MR measurements qualify as biomarkers?

From these results it seems that conventional MR is able to predict outcome at 2 years of age. The strength of these results is that even after correction for other MR variables independent MR risk factors for neurodevelopmental outcome could be determined. However, some odds ratios had quite wide confidence intervals which could be related to the small sample size within some of the MR variables: for example the OR for IVH for cognitive scores <85 was 5.1 with a 95% CI of 1.2 to 22.1. Therefore IVH might not accurately predict outcome. Conventional MR findings rely on qualitative subjective visual assessment. Assessment of DEHSI is discussed as an example: DEHSI relies on visual assessment of T2 weighted images. Image signal intensity can be scaled individually by the radiologists or neonatologists and this scaling will affect the scoring of the images. The literature reports good (Counsell et al. 2003) and poor interobserver agreement (Hart et al. 2010) in the assessment of DEHSI. This might explain the varying incidences of DEHSI in the literature: incidences between 53% (Hart et al.) and 75% (Maalouf et al. 1999) in similar populations are reported. In this cohort, the incidence of DEHSI was 77.5% in agreement with Maalouf et al. (Maalouf et al. 1999). DEHSI was only predictive for expressive language outcome in this study; hence, its usefulness as a biomarker is doubtful. Other MR variables such as cPVL, HPI, PWML or cerebellar haemorrhages are more obvious MR diagnoses and independent of signal intensity scaling, and hence more reliable. cPVL is known to predict poor outcome; hence, cPVL could serve as a biomarker. However the incidence of cPVL is very low and hence, this would be applicable for only a very small number of infants. The same problem applies for HPI and cerebellar haemorrhage. For future neuroprotective studies in preterm infants a biomarker should be found which serves as a surrogate endpoint in all preterm infants independent of visible brain injury on MR such as for example HPI or PWML. Quantitative MR measurements such as T2, ADC or FA might fulfil all criteria of such a biomarker: they are all objectively measureable, and can detect a

particular effect; however whether they can serve as surrogate endpoints for neurodevelopmental outcome is under investigation. In this study, all quantitative MR measurements were derived from ROIs positioned by one observer. Although in this as in other studies (Counsell et al. 2003) good intra-and interobserver agreement in ROIs placement could be shown, there remains a small risk of introducing observer error in ROI placement. This small observer error could be avoided by using an automated voxel and group-wise observer independent whole brain approach such as TBSS, which aligns FA images from multiple subjects. Although it is termed whole brain approach, by predefining a FA threshold one limits FA analyses to major white matter tracts: grey matter structures relating to neurodevelopmental outcome cannot be analysed with such an approach. The advantages of an ROI approach is that many brain regions can be analysed, it can be applied with a range of different MR techniques and no additional software or training is required to obtain these measurements; hence, it is an approach which can easily be introduced into clinical practice. T2, ADC and FA are objectively measurable.

Is there firm evidence from this study that T2, ADC or FA to qualify as surrogate endpoints in future neuroprotective interventional studies in preterm infants?

Ideally, this biomarker should be a surrogate endpoint for cognitive, motor and language outcome. Alternatively, different biomarkers are chosen, one for each outcome measure. As T2 relaxometry can be obtained within approximately 2 minutes of scanning time, and it is not as prone to movement artefacts as longer DTI sequences, and therefore it is a clinically easy applicable sequence in this cohort. T2 values showed regional variability within preterm infants and these correlated with cognitive, motor and language outcome at two years of age, even after multiple corrections for clinical and MR variables. Hence, T2 was predictive even in infants without cystic WM injury. The regions in which T2 correlated with neurodevelopmental outcome are known predilection areas for white and grey matter injury and their correlation with outcome can be explained by the function of the involved brain area. However, T2 reference values need to be evaluated for each ROI as there is regional T2 variability. One limitation is that T2 in only two ROIs remained independent risk factors for outcome in multiple linear regression. A sample size calculation based on 80% power, a significance level of 0.05 and an effect size of 15% and with T2 as predictor showed a sample size of 67 participants. 80 infants were imaged, however only 54 attended 2 year FU. Hence, maybe if more infants would have had 2 year FU more significant correlations between T2 and outcome could have been found. Or when automated T2 analyses are performed in

the same cohort these results might be confirmed and strengthen the qualification of T2 as a biomarker. Hence, T2 seems a promising biomarker but further analyses must be done before its implementation.
There is less evidence in this cohort for ADC to qualify as a biomarker compared to T2 as its correlation with outcome was less prominent. Splenium FA and eigenvalues showed good correlation with cognitive and language outcome; however no correlation was found with motor outcome on binary stepwise regression. The lack of correlation between FA and motor outcome might also be explained by the small sample size or the early age of neurodevelopmental assessment. Interestingly, in the study by Drobyshevsky et al PLIC FA of the first scan (performed at 10-14 days of life) correlated with motor outcome however PLIC FA measured at 36 weeks did not. The correlation between FA and outcome was strong and splenium FA might serve as a biomarker for language and cognitive outcome. Furthermore, small corpus callosum had a similar correlation with outcome to that of splenium FA; this can be explained by altered microstructure shown by FA leading to abnormal callosal size.

From this data no conclusion can be drawn as to whether quantitative MR measures serve as better biomarkers than qualitative MR findings. Both techniques give us some information about the outcome at two years of age. Maybe based on this data, a combination of information of both techniques should be used to help counselling the parents. For example, a small CC on conventional MR in combination with low FA will firm the evidence and inform the clinicians about the risk for later neurodevelopmental impairment.

As mentioned in the discussion, volumetric measurements in combination with quantitative MR measures such as FA, ADC or T2 would have been interesting to assess for correlation with outcome and these volume changes could have been put in relation with FA, ADC or T2 changes. However, as no such support was given during the time of this thesis, these analyses will have to be done as when they are available. What will be even more informative is to assess this cohort at a later age when more specific cognitive testing can be done and then to evaluate the correlation of volume and FA/T2/ADC changes with outcome.

Chapter 6

6. Future work

Methods:

The quantitative MR analysis (ADC, T2, FA/eigenvaues) of this present study was based on a ROI approach. Such an ROI approach is more objective than visual assessment however there remains an aspect of subjectivity in placement of ROIs. Although we and many other groups that use the ROI approach could show good interobserver agreement, an automated approach is more objective, more reproducible and more usable for comparison between groups, and hence more reliable. However, an approach like TBSS has its limitations. By thresholding the data to FA values one might choose to confine the analysis only to major white matter tracts and there is a risk that some regions with low anisotropy will be missed. Voxel-based morphometry, another automated approach, requires a step of tissue classification that can pose a problem in the neonatal brain with predominantly unmyelinated white matter.

A robust, reproducible and reliable MR biomarker will give us the opportunity to monitor future neuroprotective intervention. In term infants after perinatal encephalopathy MRS provides a robust biomarker as it has been shown to be predictive for neurodevelopmental outcome (Thayyil et al. 2010) and TBSS has been proposed as an MR biomarker for the detection of treatment effect (Porter et al. 2010). As T2 analysed by the ROI approach has been shown to be predictive for outcome, it would be interesting to evaluate the T2 data with an automated approach

1. Therefore, one of my future research aims is to develop an automated whole brain voxel-based approach in analysing T2 data. This will enable us to analyse T2 observer independently and will allow coverage of more brain tissue than by the ROI approach and hence, correlation with outcome might be clearer. Another alternative would be to draw larger ROI with histograms; however this approach will be as time consuming as drawing ROI and less objective.
2. As these data have been acquired with only six directions a more automated approach like TBSS is not possible
3. In future MR studies I would include MRS of the WM, especially if sequential MR will be done to see whether altered FA/T2 relate to a specific biochemical profile

As many volumetric MR studies showed abnormal brain volume in preterm infants compared to term infants or compared within preterm infants, it would be interesting to analyse this cohort with either deformation based morphometry or with the

volumetric approach suggested by Inder et al.(Inder et al. 2005) During the duration of this thesis work no volumetric software and support was available to analyse the acquired 3D FLASH data; hence, the data still needs to be analysed as it would be of interest to see whether prolonged T2 and low FA relate to regional volume changes.

Timing of MRI:

For practical reasons MR was done at term equivalent age. However, to assess brain maturation and to evaluate the effect of clinical parameters or neuroprotective treatment on brain growth it is mandatory to perform sequential scanning to be able to evaluate the best timing of scanning to assess the trajectory of brain growth

The next project will assess brain maturation with T2, ADC, FA, WM MRS and volumetric analysis with sequential scanning to evaluate when changes in T2 and FA occur and whether the infants with altered FA and T2 will have a different brain growth trajectory/ biochemical profile compared with those with normal FA/T2 for gestational age and whether these changes correlate with outcome.

Follow-up:

The infants of this cohort have been assessed at two years, which is an early but very practicable time point for assessment. However, detailed higher cognitive functions such as executive functions are better tested at later age. One consideration is that especially in cities like London with a high migration rate it is very difficult to study these infants longitudinally as high drop out is expected unless there is a research programme with a dedicated follow-up coordinator, FU specialists including neuropsychologists and research funding. It is essential to assess these infants at a later age to better delineate cognitive (especially executive functions) and behavioural function and their correlation to early imaging. Furthermore it would be most informative to perform another MR at the same time to assess the evolution of the observed early changes and to evaluate their long-term effects.

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Appendix 1

Data entry for perinatal minimal data set
For all infants < 29 weeks and/or ≤ 1,000g

Name

Date of birth **... ... /... ... / 20....**

Hospital number ……

surveyno ……

OBS.: Use codes 8, 88, 888, or 8888 for not applicable information.

Use codes 9, 99, 999 or 9999 for unknown information.

gest

Absolute value in days (weeks x 7 + days) (__ __ __)

sex

Female (1)

Male (2)

bw

Absolute value in g (__ __ __ __)

gestcent

<0.4th (1)

>0.4th – 2nd (2)

>2nd – 9th (3)

10th – 90th (4)

>90th (5)

hospborn

UCH born (1)

Ex-utero transfer (2)

multpreg

Singleton (1)

Twin 1 (2)

Twin 2 (3)

Triplet 1 (4) etc

race

Caucasian	(1)
Black	(2)
Asian	(3)
Other (incl mixed ethnicity)	(4)
Don’t know	(9)

matage	**Maternal age**
absolute value in years	(__ __)
don’t know	(99)
mateduc	**Maternal education**
Left school <17 yrs	(1)
Left school >17<19yrs	(2)
Higher education; degree or diploma	(3)
Not finished	(4)
Never went to school	(5)
Don’t know	(9)

ocupynow

Going to school	(1)
Paid employment or self-employed	(2)
Looking for work	(3)
Looking after the home and family	(4)
Something else	(5)
Don’t know	(9)

ocupydad

Going to school	(1)
Paid employment or self-employed	(2)
Looking for work	(3)
Looking after the home and family	(4)
Something else	(5)
Don’t know	(9)

soclas01

Not known	(99)

smoking	**Maternal smoking**

Ave no. cigarettes smoked per day	(__ __)	
Stopped smoking when pregnant		(88)
Don't know		(99)
alcohol		
Average number of units per week	(__ __ __)	
Never drank alcohol		(888)
Don't know		(999)
ilegdrug		
Yes		(1)
No		(2)
Don't know		(9)
llegdrug2		
Opiates		(1)
Cocaine		(2)
Cannabis		(3)
Benzodiazepine		(4)
Combination		(5)
Not applicable		(8)
Don't know		(9)
assist		
No		(2)
Yes – drugs only		(1)
Yes – IVF/GIFT etc		(3)
pet		
Yes		(1)
No		(2)
Yes plus fits		(3)
Don't know		(9)
highbp		
Yes		(1)
No		(2)
Don't know		(9)
prevpreg		
Number		(__ __)

Don't know (99)

miscar

Number (__ __)

Never had (888)

Don't know (999)

stilbpre **Stilbirths at less than 37/40**

Number (__ __ _)

Never had (888)

Don't know (999)

stilbter **Stilbirths at ≥37/40**

Number (__ __ _)

Never had (888)

Don't know (999)

prem1 Previous preterm delivery at <32/40

Number (__ __ _)

Never had (888)

Don't know (999)

prem2 Previous preterm delivery at ≥32/40 and ≤37/40

Number (__ __ __)

Never had (888)

Don't know (999)

hep Low molecular weight heparin during pregnancy

Yes (1)

No (2)

Don't know (9)

warf **Warfarin**

Yes (1)

No (2)

Don't know (9)

MgSo **Magnesium sulphate**

Yes (1)

No (2)

Don't know (9)

NSAID **NSAIDS related to delivery.**

Yes	(1)
No	(2)
Don’t know	(9)
asp	**Low dose aspirin**
Yes	(1)
No	(2)
Don’t know	(9)
otherdrg	**Other drugs**
Yes	(1)
No	(2)
Don’t know	(9)
antihyp	**Antihypertensive drugs to mother**
Yes	(1)
No	(2)
Don’t know	(9)
antibio	**Use of antibiotics within 2 weeks prior to delivery**
Yes	(1)
No	(2)
Don’t know	(9)
antitoco	**Use of anti tocolysis**
No	(1)
Ritodrine	(2)
Salbutamol	(3)
Nifedipine	(4)
Atosiban	(5)
Combination of above	(6)
Other	(7)
Don’t know	(9)
matill	**Pregnancy induced or chronic maternal illness.**
No	(01)
Diabetes	(02)
Antiphospholipid syndrome	(03)
SLE	(04)
Renal failure	(05)

Cardiac disease	(06)
Thyroid disease	(07)
Epilepsy alone	(08)
Neurological disease	(09)
Other	(10)
Combination	(11)
Don’t know	(99)

famhist **Family history**

No	(01)
Hereditary disease –	
Metabolic hereditary disease	(02)
Chromosomal	(03)
Epilepsy	(04)
Coagulopathy/prothrombotic disease	(05)
Febrile seizures	(06)
Neonatal seizures	(07)
Behavioural disorder	(08)
Learning disabilities	(09)
Psychiatric disorder	(10)
Cardiovascular disease	(12)
Combination	(11)
Don’t know	(99)

fmu **Scanned after 20 weeks gestation**

Yes	(1)
No	(2)
Don’t know	(9)

liqvol **Liquor volume**

Normal	(1)
Increased	(2)
Decreased	(3)
Not done	(8)
Not known	(9)

iuproc **Intra- uterine procedures**

None (1)

Known to Fetal Medicine Unit (2)

Not known to Fetal Medicine Unit (3)

Don't know (9)

matfever **Maternal fever >38^0**

None (1)

Within 24 hours of delivery (pre or post) (2)

Within 2 weeks prior to delivery (3)

Don't know (9)

crpabs **Highest CRP in pregnancy (within 2 weeks prior to delivery)**

Absolute value (__ __ __)

Not done (888)

Don't know (999)

mwccabs **Highest maternal white cell count/1000 (within 2 weeks prior to delivery)**

Absolute value (000)

Not done (888)

Not known (999)

msu **MSU result (within 2 weeks prior to delivery)**

Candida (1)

Group B strep (2)

Other (3)

No growth (4)

Not done (8)

Don't know (9)

hvs **High vaginal swab results**

Candida (1)

Group B strep (2)

Other (3)

No growth (4)

Not done (8)

Don't know (9)

plhistol **Placental histology**

Normal		(01)
Chorioamnionitis		(02)
Funisisis		(03)
Chorioamnionitis and funisitis		(04)
Infarcts		(05)
Excess fibrin+/- syncitial knots		(06)
Infarcts + (6)		(07)
Other		(08)
Not done		(88)
Don’t know		(99)
concmv	**Maternal congenital infection – CMV**	
Yes		(1)
No		(2)
Not done		(8)
Don’t know		(9)
contoxo	**Maternal congenital infection – Toxoplasmosis**	
Yes		(1)
No		(2)
Not done		(8)
Don’t know		(9)
conhepb	**Maternal congenital infection – Hepatitis B**	
Yes		(1)
No		(2)
Not done		(8)
Don’t know		(9)
conhepc	**Maternal congenital infection – Hepatitis C**	
Yes		(1)
No		(2)
Not done		(8)
Don’t know		(9)
conrube;	**Maternal congenital infection – Rubella**	
Yes		(1)
No		(2)
Not done		(8)

Don’t know		(9)
convdrl	**Maternal congenital infection – VDRL**	
Yes		(1)
No		(2)
Not done		(8)
Don’t know		(9)
conhiv	**Maternal congenital infection – HIV**	
Yes		(1)
No		(2)
Not done		(8)
Don’t know		(9)
conherp	**Maternal congenital infection – Herpes**	
Yes		(1)
No		(2)
Not done		(8)
Don’t know		(9)
conoth	**Maternal congenital infection – other**	
Yes		(1)
No		(2)
Not done		(8)
Don’t know		(9)
pp	**Placenta previa**	
Yes		(1)
No		(2)
Don’t know		(9)
abrupt	**Abruption**	
Yes		(1)
No		(2)
Don’t know		(9)
bleeding	**Antepartum haemorrhage**	
1st trimester only		(1)
2nd trimester only		(2)
3rd trimester only		(3)
1st and 2nd		(4)

1st and 3rd		(5)
2nd and 3rd		(6)
Throughout pregnancy		(7)
No		(8)
Don't know		(9)
prom	**Rupture of membranes prior to onset of labour**	
<24 hours		(1)
24 – 48 hours		(2)
>48 – 72 hours		(3)
>72 hours		(4)
No rupture		(8)
Not known		(9)
labonset	**Onset of labour**	
Spontaneous		(1)
Induced		(2)
No labour		(8)
Don't know		(9)
ctg	**Cardiotography**	
Normal		(1)
Variable decelerations		(2)
Late decelerations		(3)
Bradycardia<110		(4)
Tachycardia		(5)
Flat unreactive	/sinusoidal	(6)
Not done		(8)
Not known		(9)
analges	**Analgesia in labour**	
Inhilational only		(1)
Opiates only		(2)
Inhilational plus opiates		(3)
Epidural/spinal alone		(4)
Epidural plus inhalational or opiates		(5)
Not done		(6)
No labour		(8)

Don't know (9)

meconium **Meconium stained liquor**

Yes (1)

No (2)

Don't know (9)

present **Presentation**

Vertex (1)

Breech (2)

Other (3)

Not known (9)

model **Mode of delivery**

Vaginal – no instruments (1)

Instrumental (2)

LSCS in labour (3)

LSCS no labour (4)

Don't know (9)

resus **Resuscitation of baby**

Minor (suction,O_2,bag and mask, naloxone) (1)

Major (intubation, CPR, adrenaline etc) (2)

None (8)

Don't know (9)

apgar1 **Apgar at 1 minute**

Absolute value (__ __)

Don't know (99)

apgar5 **Apgar at 5 minutes**

Absolute value (__ __)

Don't know (99)

apgar10 **Apgar at 10 minutes**

Absolute value (__ __)

Don't know (99)

cordph **Cord pH**

Absolute value to two decimal places (__ .__ __)

Don't know (9.99)

cordbe **Cord base excess**

Absolute value to one decimal place (__ __.__)

Don't know (99.9)

worsttph Worst pH within 24 hours of birth

Absolute value to two decimal places (__.__ __)

Don't know (9.99)

worstbe Worst base excess within 24 hours of birth

Absolute value to one decimal place (__ __.__)

Don't know (99.9)

Highlact Highest lactate within first 24 hours

Absolute value to two decimal places (__ __.__ __)

Don't know (99.99)

ofc Head circumference at birth

Absolute value in cm to 1 decimal place (__ __. __)

Don't know (99.9)

congmalf Congenital abnormality

Neurology (01)

Heart (03)

Skeletal (04)

GIT (05)

Chromosomal anomaly (06)

Other recognised syndromes (07)

Unclassified syndrome (08)

Urogenital anomaly (10)

None (88)

Don't know (99)

neocrpno Highest CRP in first three days of life

Absolute value (__ __ __)

Not done (999)

Don't Know (888)

neopltno Lowest platelet count in first three days of life

Absolute value (__ __ __)

Not done (999)

Don't Know (888)

neobc Blood cultures in first three days of life

Negative (1)

Positive (2)

Not done (999)

Don't Know (888)

neomen Proven meningitis at any time ie positive lumbar puncture

Yes (1)

No (2)

Not done (999)

Don't Know (888)

prosta Prostacyclin/prostaglandin/ tolazoline/ nitric oxide

Yes (1)

No (2)

Don't know (9)

inotropes Inotrope group of drugs

Yes (1)

No (2)

Don't know (9)

conginf Congenital infection screen done ie CMV, Toxo, Rubella etc.

Yes positive (1)

Yes negative (2)

Not done (8)

Don't know (9)

timefeed Time to establish enteral feeding

Absolute value days (__ __ __)

Feeding not fully established at discharge (888)

Don't know (999)

Disfeed Feeding at discharge

Fully orally fed (1)

Fully NG/PEG fed (2)

Mixed PO and NG (3)

TPN (4)

Don't know (9)

wtdisch Weight on discharge/transfer in grams

Absolute value (__ __ __ __)

Don't know (9999)

ofdisch OFC on discharge/transfer in cm to 1 decimal place.

Absolute value (__ __.__)

Don't know (99.9)

locdisch Method of discharge

Home (1)

Other hospital (2)

Galaxy Ward (3)

Died (4)

Don't know (9)

agedisch Age at discharge in days

Absolute value (__ __ __)

Not discharged (died) (999)

agdeadh Age at death in hours

Absolute value in hours. (__ __ __)

More than 672 hours (888)

agdead Age at death in days if more than 28 days

Absolute value in days. (__ __ __)

Alive at discharge (888)

eeg12 12 lead EEG

age of recording in hours (__ __ __ __)

Normal (0001)

Abnormal at any time (0002)

Not done (8888)

Don't know (9999)

cfm CFM study performed

age of recording in hours (__ __ __ __)

Normal (0001)

Abnormal at any time (0002)

Not done (8888)

Don't know (9999)

ofcterm OFC at term in cm to 1 decimal place

Absolute value (__ __.__)

Not known		(88.8)
Don't know		(99.9)
termage	**Age at term neurology in days (38 -44 weeks x 7 + days)**	
	Absolute value	(__ __ __)
	Not done	(888)
	Don't know	(999)
termcns	**Term neurology (≥37 - ≤44 weeks)**	
	Optimality score	(__ __. __)
	Not done	(8.8)
	Don't know	(9.9)
mriage	**Age in days at term MRI in weeks**	
	Absolute value	(__ __ __)
	Not done	(888)
	Don't know	(999)
aabr	**Hearing tests performed - AABR**	
	Pass	(1)
	Fail right	(2)
	Fail left	(3)
	Fail both	(4)
	Not done	(8)
	Don't know	(9)
heart	**Other hearing tests performed**	
	Yes	(1)
	No	(2)
	Don't know	(9)

preterm	**Was this infant <32/40 and/or <150**	
Yes		(1)
No		(2)

Please, continue filling the form if infant <32/40 and/or <1500g. If not, mark not applicable

nirstudy	**Neonatal near infrared study performed**	
Yes		(1)
No		(2)

Not applicable (8)

Don’t know (9)

cytstudy **Enrolled into cytokine study**

Yes (1)

No (2)

Not applicable (8)

Don’t know (9)

drift **Enrolled into DRIFT trial**

Yes (1)

No (2)

Not applicable (8)

Don’t know (9)

steroids Antenatal steroids to mother for lung maturity.

Betamethasone (1)

Dexamthasone (2)

Other steroids (3)

Steroids for other reasons (4)

Mixed steroids (5)

Not done (6)

Not applicable (8)

Don’t know (9)

numroids Number of courses (not doses) of steroids

Absolute number (write 00 if not done) (__ __)

Not applicable (88)

Don’t know (99)

comroids Were steroids courses complete?

Yes (1)

No (2)

Not applicable (8)

Don’t know (9)

hmd Acute respiratory distress – surfactant deficiency

Yes (1)

No (2)

Not applicable (8)

Don’t know (9)

pneux **Pneumothorax.**

Yes (1)

No (2)

Not applicable (8)

Don’t know (9)

ventdays **Number of days ventilated by IPPV including oscillation but excluding nasal CPAP**

Absolute value (__ __ __)

Not applicable (888)

Don’t know (999)

modvent **Mode of ventilation**

Never ventilated (1)

Oscillation predominantly (>80%) (2)

Conventional predominantly (>80%) (3)

Oscillation and conventional (4)

Not applicable (8)

Don’t know (9)

ncpap **Total no. of days on CPAP**

Absolute value (if Never on CPAP write 000) (__ __ __)

Not applicable (888)

Don’t know (999)

dcpap **Was the infant discharged on CPAP**

Yes (1)

No (2)

Not applicable (8)

Don’t know (9)

jaundice **Highest unconjugated bilirubin**

Absolute value (if Not done write 000) (__ __ __)

Not applicable (888)

Don’t know (999)

pda **PDA**

Yes no treatment (1)

Yes surgery only (2)

Yes medical treatment only		(3)
Yes medical plus surgery		(4)
No		(5)
Not applicable		(8)
Not known		(9)
guts	**Proven NEC clinically or radiologically**	
Yes		(1)
No		(2)
Suspicious		(3)
Not applicable		(8)
Don't know		(9)
renal	**Significant renal failure – decreased urine O/P plus abnormal creatinine**	
Yes		(1)
No		(2)
Not applicable		(8)
Don't know		(9)
seiz	**Seizures (confirmed with EEG)**	
Yes		(1)
No		(2)
Not applicable		(8)
Don't know		(9)
Abnmov	**Abnormal movements**	
Yes		(1)
No		(2)
Not applicable		(8)
Don't know		(9)
anticonv	**Anticonvulsants**	
Yes		(1)
No		(2)
Not applicable		(8)
Don't know		(9)
Babydrug1	**Drugs while in NICU Theophylline**	
Yes		(1)

No		(2)
Not applicable		(8)
Don’t know		(9)
Babydrug2	**Drugs while in NICU Caffeine**	
Yes		(1)
No		(2)
Not applicable		(8)
Don’t know		(9)
Babdrug3	**Drugs while in NICU Ibuprofen**	
Yes		(1)
No		(2)
Not applicable		(8)
Don’t know		(9)
Babdrug4	**Drugs while in NICU Indomethacin**	
Yes		(1)
No		(2)
Not applicable		(8)
Don’t know		(9)
Babdrug5	**Drugs while in NICU Hydrocortisone**	
Yes		(1)
No		(2)
Not applicable		(8)
Don’t know		(9)
Babdrug6	**Drugs while in NICU Dexamethasone**	
Yes		(1)
No		(2)
Not applicable		(8)
Don’t know		(9)
Babdrug7	**Drugs while in NICU Inotropes**	
Yes		(1)
No		(2)
Not applicable		(8)
Don’t know		(9)

Babdrug8 **Drugs while in NICU Antireflux treatment**

Yes (1)

No (2)

Not applicable (8)

Don't know (9)

rlf **ROP noted in NNU**

grade 1-11 (1)

grade III or worse (2)

Retinal detachment (3)

No (4)

Not applicable (8)

Don't know (9)

roomair **Time to room air in days**

Absolute value (if never in air, write 000) (__ __ __)

Not applicable (__ __ __)

dischair **O_2 on discharge**

Discharged to other hospital or ward in the same hospital in O_2 (1)

Discharged home in oxygen (2)

Not applicable (8)

Not known (9)

36wko_2 **In oxygen at 36 weeks**

Yes (1)

No (2)

Not applicable (8)

Don't know (9)

28dayso_2 **In oxygen at 28 days**

Yes (1)

No (2)

Not applicable (8)

Don't know (9)

termo_2 **In oxygen at term**

Yes (1)

No (2)

Not applicable	(8)
Don’t know	(9)

Comments:

Appendix 2

MRI scoring sheet

	WM quality						
		T1w	T1w	T2w	T2w	Lobe R	Lobe L
Punctate lesions		right	left	right	left		
location	PV WM						
	deep WM						
	subcortical WM						
	corticospinal tract						
	optic radiation						
Size	<2mm						
	>2mm						
count	1-5						
	6-10						
	>10						
Cystic lesions							
location	PV WM						
	deep WM						
	subcortical WM						
	corticospinal tract						
	optic radiation						
Size	<2mm						
	>2mm						
focal							
diffuse							
DEHSI	Yes						
	No						
	WM volume						
Ventricles							
size	normal						
	moderate enlargement						
	marked enlargement						
measurements of VI							
irregular borders	Yes						
	No						
Corpus callosum							
size	normal						

	small						
	enlarged						
	Grey Matter						
Cortical folding	normal						
	abnormal						
ECS							
size	normal						
	mild-moderate						
	severe enlargement						
DGM (cystic, small, hemorrhage)		right	left				
	NC						
	Th						
	GP						
	Putamen						
	Myelination						
		Right	left				
Corticospinal tract	sup. To PLIC						
	PLIC						
	inf. To PLIC						
Dorso brainstem							
Thalamus ventrolat.							
Putamen ventrolat.							
	Cerebellum						
SI		normal	abnormal	increased			
WM SI							
GM SI							
Focal lesions	Yes						
	No						
location							
Size	Normal						
	abnormal						
	Haemorrhages						
	right	left					
GMH							
IVH							
Subdural							
Epidural							
Subarachnoidal							
Lobar							

Appendix 3

In the background imaging studies relating to outcome were discussed with focus on those, which related to the aims and hypothesis of the thesis. Below are some more interesting studies, which correlate to outcome in children born preterm.

Imaging studies correlating to IQ

In a voxel-based morphometry study of 309 healthy children and adolescents a relationship between increasing IQ and increasing cortical thickness for the older children, adolescents and young adults was found (Fig.1.52) (Shaw 2007). However, the inverse relationship was found for the youngest group, in whom IQ was negatively correlated to cortical thickness which implies a link that is highly sensitive to development (Shaw 2007). The trajectory linked to intelligence was most pronounced in the frontal lobes, the cortical regions of late structural and metabolic maturation.

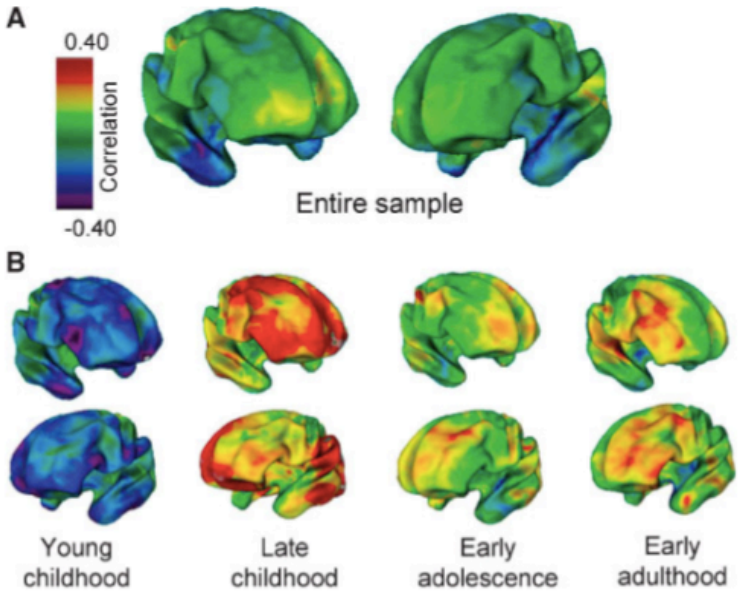

Fig.1.52. Correlation between IQ and cortical thickness. A. Pearson's correlation for all 307 subjects was modestly positive. B. Correlations in the different age group showing negative correlations in the youngest group indicating that higher IQ was associated with thinner cortex, particularly in frontal and temporal cortex (Shaw 2007)

This is an interesting finding in the context of the high incidence of cortical abnormalities found in preterm infants which would set them on a different growth trajectory and hence, IQ development.

Imaging studies correlating to language outcome

A functional MRI study showed that 8 years old preterm children seemed to process meaningful speech in the same pathways that term children processed strings of meaningless sounds, the more this was true for preterm children, the poorer was their comprehension of the meaning of the story and the poorer were their verbal subscale and verbal comprehension IQ scores (Peterson et al. 2002). During the semantic processing task, activity in Wernicke's area and in all of its component Brodmann's areas correlated inversely with verbal comprehension IQ and story comprehension IQ. These findings suggest that the preterm children who are particularly cognitively impaired may not have made sense of the story because they tended to hear the normal story more like term children heard the randomised story: meaningless, phonemic sounds (Peterson et al. 2002)

Imaging studies correlating to autism spectrum disorder

In 1999, a voxel-based whole brain study identified grey matter differences in the amygdala and the cerebellum in 15 years old adolescents with autism (Abell et al. 1999). Neuroanatomical differences between children with ASD and controls infants were reported showing different brain growth pattern with greater total brain volume that appears during first few years and disappears from adolescence onwards (Bolton et al. 2001; Courchesne et al. 2001; Aylward et al. 2002). Recent paediatric and adolescent neuroimaging studies report regional increase of grey matter ((fusiform gyrus, cerebellum, prefrontal cortex, peri-hippocampal cortex, occipitotemporal sulcus and cortical thickness in temporal and parietal lobes respectively) (Salmond et al. 2005; Hardan et al. 2006) accompanied with local reduction of white matter (Chung et al. 2004); these findings are in keeping with previous published work showing abnormal brain growth in children with autism (Redcay and Courchesne 2005). In a recent voxel-based morphometry study increased regional grey matter and decrease in regional white matter was found in patients with mean age of 12.4 years with autism (Bonilha et al. 2008). Proton MRS studies of autism have shown regional-specific reductions of grey matter NAA (Friedman et al. 2003).